Extraction of oil from fish processing waste for fuel applications: Process development, analysis and feasibility

By

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Abstract

The fish processing industry generates a significant amount of fish byproducts that could either be an important source of energy, food, or industrial feedstock. Fish oils are made up of several lipid classes. These lipids contain long-chain omega-3 polyunsaturated fatty acids; PUFA, (mostly eicosapentaenoic acid (EPA: C20:5 n-3), and docosahexaenoic acid (DHA: C22:6 n-3)) and other lipid classes. The traditional market for the nutritional lipids in the fish oil has been the food industry. However, significant infrastructure for the strict quality control systems is required for food grade oils. Therefore, the process of extracting the high nutritional oils is energy intensive. Further, the location and infrastructure limit the feasibility due to processing requirement, storage and/or transportation issue. For biofuel implementation, the processes to extract and refine fish oil are less energy intensive than the processes for nutritional quality oils and do not require the stringent product specifications and approval process as in the food and pharmaceutical industry. There have been significant advances in developing inexpensive and robust methods for fuel extraction and upgrading processes.

Supercritical fluid extraction process using carbon dioxide (SC-CO_2) under moderate conditions is promising for quality fish oil production. This process produces oil with low impurities compared to other processes. The specific research objectives include: (1) Overview and background information on Atlantic Canada fish processing plants (2) Review of (state of art) fish oil extraction processes including physical, chemical and biological processes. (3) Fuel oil characterization of fish oil and fish oil blends with petroleum distillate. This section evaluates thermal stability, rheological, and heating

values, of crude fish oil and blend with heavy petroleum distillate. (4) Solubility determination of fish oil in SC-CO_2; the SFE extraction process (optimize the SFE process conditions to maximize fish oil yield under the least intensive conditions, pressures, temperatures and CO_2 consumption); and mathematical model to predict oil extraction rate as a function of process conditions. (5) Fuel oil quality evaluation as a function of extraction methods. (6) Life cycle assessment (LCA) of different extraction processes (modified fishmeal process (MFM), SC-CO_2, and soxhlet processes) of fish oil from salmon wastes. The environmental burdens and potential impacts by each extraction process were quantified through the LCA.

In the characterization phase of this study, the thermal and rheological properties of unrefined salmon oil, bunker fuel oil and their blends have been analyzed. The feasibility of using unblended and/or blends of fish oil in conventional heaters/boilers/engines are determined by these properties. The MFM oils, the bunker fuel oil and their blends behaved as a shear thinning non-Newtonian fluid that can be described by the power-law model.

The supercritical carbon dioxide extraction (SC-CO_2) was effective at pressures of 15, 25, and 35 MPa, temperatures of 313, 333 and 353 K, and CO_2 flow rates of 0.18-0.48 kg/hr. The process is solubility controlled and the yields at 35 MPa, temperatures of 313, 333 and 353 K, and CO_2 flow rates of 0.18 kg/hr were approximately 39, 46 and 41 (wt.%). The mathematical model (Goto et al. 1993) using the best fit of theoretical extraction curve correlated the experimental data satisfactorily with average absolute deviation, AAD (%) ranged from 2.4 to 10.6 %. Unlike the MFM oil, the SC-CO_2 oil is more

viscous homogenous oil which behaved as a Newtonian fluid. Physco-chemical, compositional and thermal characterization indicated SC-CO_2 oil contains fewer impurities than the MFM and soxhlet process oils.

From the LCA results, the MFM method has the lowest overall environmental impact compared to the soxhlet and SC-CO_2 methods. The soxhlet extraction method, due to the use of solvent (hexane), has the greatest impacts on all of the four damage categories (human health, ecosystem quality, climate change and resources). The SC-CO_2 requires more energy to produce 1 kg fish oil (2 folds more than the MFM and 3 folds more than the soxhlet methods). However, the SC-CO_2 produces higher quality oil than the other two processes and the process impact on the environment is moderate relative to the MFM and soxhlet processes.

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CHAPTER 1

1.0 INTRODUCTION AND OVERVIEW.

The Canadian fish processing industry is one of the major seafood and marine product exporters, with approximately 75 % fish products exported to over 80 countries. Atlantic Canada (Fig.1.1) was about 40,000 km of coastline which comprises four provinces including Newfoundland and Labrador, New Brunswick, Prince Edward Island and Nova Scotia. In 2011, the total sea fisheries landed in Canada was 850,533 metric tons with 703,905 metric tons (82.76%) from the Atlantic Region [1]. Before the final sale, approximately 70% of the fish landed is processed [2] resulting in a considerable percentage (20 – 80% wt) of fish waste depending on the processing extent and fish type [3]. Fish discards may include the heads, frames, trimmings, fins, skin and viscera. Some of the by-products are used while the main bulk is considered waste and dumped into the sea or landfilled, creating both disposal and pollution problems [2]. In Atlantic Canada, fish processing industries generate approximately 418,000 tons of fish wastes annually [3]. Newfoundland and Labrador (NL) processing plants (excluding seal processing plants and fish meal plants) generate fish wastes in the amount of 35,000 tons annually [3], which are disposed into the oceans. The most commonly processed species are capelin, herring, mackerel, seal and farmed salmanoids [4]. The disposal of fish waste in the ocean leads to considerable oxygen shortage in the water due to activities of the aerobic bacteria on the organic matter in the presence of oxygen.

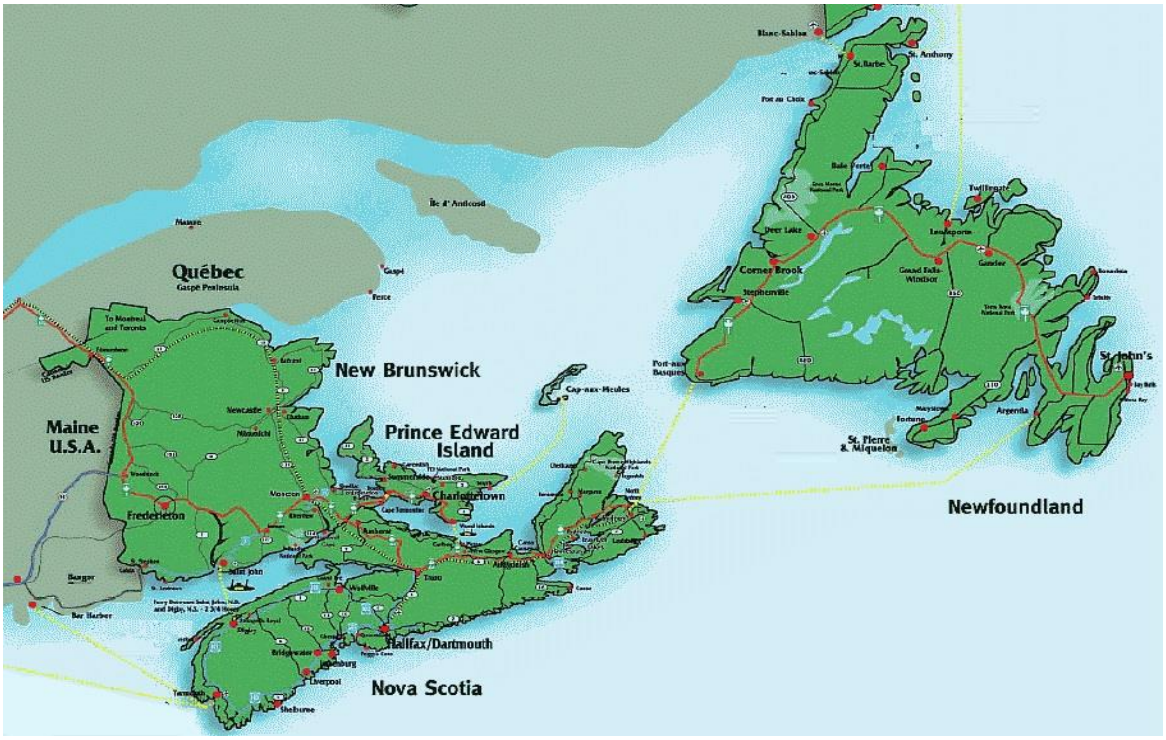

Figure 1.1: Atlantic Canada. (Sourced from Goggle)

Fish waste also causes overloads of nitrogen, phosphorous and ammonia, which can lead to pH changes and increased water turbidity, which can also lead to the decomposition of algae [1]. Fish processing waste contains valuable by-products which may include; fish oil (ω-3 fatty acids), proteins and amino acids, chitosan, chitin, collagen and gelatin, cosmetics, natural pigments, enzymes, animal feed, and soil fertilizers [1,5]. In areas of intense fish processing, these by-products are further processed into fishmeal where the byproduct is waste fish oil.

The fish oil fatty acids (PUFA; DHA and EPA) are known for their significant roles in human health and nutrition such as prevention and treatment of coronary heart disease, blood platelet aggregation, hypertension, arthritis, abnormal cholesterol levels, mental illness and autoimmune disorders [6,7]. Fish oil are also a rich source of vitamins

including vitamin A, D, E and K, which are soluble content of the oil and need to be extracted for human consumption due to their significant roles in human health and metabolism. On an annual basis, EPA demand is about 125 tonnes in Japan and demand is much greater world-wide for nutritional supplement [6]. PUFA occurs as triacylglycerides (TAG) in fish oil at levels between 10 and 25 % and resulting market growth has necessitated much interest in PUFA extraction and the concentrating method from natural sources [7].

However, due to degradation or low quality, waste derived fish oils are not suitable for edible oils and could serve as a possible low grade fuel. The fish oils when compared to petroleum based fuels have the advantage of lower toxicity, higher biodegradation rates (reducing impact on soil and water if spilled), no sulfur, and a higher flash point [8, 9, 10, 11]. The extraction of fish oil (edible and non-edible) can be done through several methods using physical, chemical and biological approaches. The properties which make fish oil a replacement/blend option to petroleum fuel distillates include high calorific/heating value, biodegradability, and comparable combustion efficiency. However, using unrefined fish oil in engines can cause operational issues due to composition/property differences between crude fish oil and conventional diesel fuel. Crude fish oil contains a variety of impurities such as free fatty acids, primary oxidation products, minerals, pigments, moisture, phospholipids, and insoluble impurities that reduce the oil quality. The level of the impurities present in the oil depends on the fish oil extraction method. A series of refining processes are normally used to remove these impurities, such as degumming, deodorization, bleaching, and neutralization. However,

the operating costs associated with these refining steps and loss of oil quality during processing added to the overall cost of the process. Careful selection of the initial extraction process can produce oils that contain fewer impurities and thus reduce the need for further processing of the oil. There are several challenges with this type of work, predominantly in the heterogeneity of the waste material as fish species and degree of fish processing varies based on location and season. Currently, most researchers focus on refining and conversion of crude bio-oils (plant and animal) to biodiesel using chemical, thermal, and enzymatic processes. There is limited literature comparing oil extraction processes from fish or fish waste as a function of extraction processes. The fuel properties of fish oil and petroleum fuel blends have not been studied in detail. In addition, the overall feasibility of extraction processes of oil from waste has not been studied. To address these and other issues related to fish oil extraction from fish waste, the following studies are required; various extraction processes of lipid/oil from fish residues, quality of oil derived as function of extraction processes, characterization of fish oil blends with mineral base fuels, and the environmental impacts of the extraction processes.

In this research, physical, chemical and biological extraction processes were reviewed and based on the review three processes compared; a modified fishmeal process, the traditional soxhlet extraction process, and supercritical extraction using carbon dioxide. The thesis consists of a series of manuscripts either published, revised for publication, in review processes or to be submitted for publication.

- Chapter 2 has been published in the *Biomass & Bioenergy Journal* and the manuscript provides the review of literature on the current state of art on fish oil extraction from fish. The chapter (chapter 2) describes the oil extraction methods available and proposed the effects of each method on lipid and protein products.
- Chapter 3 describes the MFM process and characterization studies on the blend of fish oil with mineral oil (heavy petroleum distillate). The manuscript has been re-submitted to the *Fuel Journal* after revision.
- Chapter 4 is divided into three sections: determination of fish oil solubility in SC-CO_2; optimization of the SFE extraction; and development of a mass transfer model to predict oil extraction as a function of process conditions. The manuscript from chapter 4 has been revised based on reviewers' comments and re-submitted to *The Journal of Supercritical Fluids* for publication.
- Chapter 5 has been accepted to the *Fuel Journal* and summarizes the evaluation of quality of the oil extracted using the three proposed extraction methods. The oil quality was compared based on lipid compositions, physico-chemical, thermal, rheological, and chemical properties.
- Chapter 6 compares the extraction processing using life cycle analysis (LCA). The energy, material and resources consumptions of the extraction methods were analyzed using SimaPro 7.
- Chapter 7 contains summary, conclusions, and recommendations.

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CHAPTER 2

LITERATURE REVIEW

A REVIEW OF LIPID EXTRACTION FROM FISH PROCESSING BY-PRODUCT FOR USE AS A BIOFUEL

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Abstract

Fish processing facilities generate a significant amount of fish by-products that could be an important source of energy, food, or industrial feedstock. While fish oil is a natural source of omega-3 polyunsaturated fatty acids (mostly eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) used in nutritional supplements, the ability to extract, refine, and get to market of these oils may be challenging at processing facilities where there is limited infrastructure and plants are remotely located. Under these conditions, extraction of oil from fish by-product for use as an in-house or regional fuel may be both economically and environmentally be a more sustainable approach. Processes to extract and refine fish oil for fuel are less energy intensive than the processes for nutritional quality oils and do not require the stringent product specifications and approval process as in the food and pharmaceutical industry. Unlike food crops, extraction of oil from fish residue does not negatively impact food production. This paper presents an overview of developments made in fish oil extraction methodologies including physical, chemical and biological processes.

A version of this paper has been published in the *Biomass and Bioenergy Journal*. The lead author is Ibraheem Adeoti and co-author is Dr. Kelly Hawboldt. Mr. Ibraheem Adeoti wrote the paper and performed all literature searches required for background information while Dr. Hawboldt provided technical guidance and editing of the manuscript.

Nomenclature

ASE	Accelerated solvent extraction
B&D	Bligh and Dyer
CO_2	Carbon dioxide
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
HPLC	High-performance liquid chromatography
MAE	Microwave assisted extraction
Mt	Million tonne
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PUFA	Polyunsaturated fatty acid
SC-CO_2	Supercritical carbon-dioxide
SCFE	Supercritical fluid extraction
SFA	Saturated fatty acid
TAG	Triacyglycerol
TG	Triglycerides

2.0 Introduction

Fish oils are a rich source of polyunsaturated fatty acid (PUFA), in particular the n-3 series *cis*-5, 8, 11, 14, 17-eicosapentaenoic acid (EPA) and *cis*-4, 7, 10, 13, 16, 19-docosahexaenoic acid (DHA). These fatty acids are used in the prevention and treatment of coronary heart disease, blood platelet aggregation, hypertension, arthritis, abnormal cholesterol levels, mental illness and autoimmune disorders [1,2]. Fish oils are also a rich source of vitamins including vitamin A, D, E and K [3]. Polyunsaturated fatty acid (PUFA) occurs as triglycerides (TG) in fish oil with a mass fraction between 10 % and 25 % [2].

Several methods have been used to concentrate PUFA in marine oils achieving different levels of recovery. Without initial hydrolysis, concentration of up to 30 % EPA and DHA can be achieved using solvent fractionation, winterization, and molecular distillation [2]. However, higher levels of concentration (65 % to 80 %) are achievable with combination of either esterification or hydrolysis with methods such as supercritical fluid extraction, urea complexation, and molecular distillation. Recoveries of above 90 % PUFA have been attained using high-performance liquid chromatography (HPLC) [2]. A number of processes have also been reported to concentrate PUFA with less or no saturated fatty acid (SFA) such as low temperature crystallization, distillation, and enzymatic enrichment [4].

Bio-oils from biomass are increasingly being used either as stand-alone or in blends with petroleum based fuels. Bio-oils composition and thermal and physical properties are a function of the feedstock and the degree of processing of the crude bio-oil. The most

common application is biodiesel, which when derived from animal and plant matter consists of fatty acid methyl ester (FAME). When derived from virgin crops, the high value of these crops requires tens of millions of liters of biodiesel to be produced annually for an industrial plant to remain economical [5]. In addition, the food versus energy debate and other environmental concerns over cultivation and conversions can limit the choice of crops for energy over food [6]. Boyd et al. [5] also reported that biodiesel manufacturing from bio-oils is not only economically more attractive than virgin crops but also results in environmental benefits. Therefore, attention has been shifted to recycled yellow and brown greases from foodservice establishments, fish oils from the fish farming and processing industries, and rendered animal fats from the agricultural industries.

Properties that make fish oil a replacement/blend option to petroleum fuel distillates are high calorific/heating value, biodegradability, and comparable combustion efficiency. However, using unrefined fish oil in engines can pose some problems due to poor flow properties especially at low temperature, lower lubricity, higher viscosity, and higher acidity compared to conventional diesel fuel [7]. This is because unrefined fish oil contains impurities such as free fatty acids, primary oxidation products, minerals, pigments, moisture, and phospholipids [8]. The presence and/or quantity of impurities depend on the fish oil recovery method [8]. Hence, careful selection of the initial extraction process can produce oils that contain fewer impurities and thus reduce the need for further processing of the oil.

Several refining processes are normally required to improve fish oil quality such as degumming, deodorization, bleaching, and neutralization. Various studies have studied the use of fish oil as fuel oil for convectional combustors or diesel engines [9,10]. Engines that use low quality fuels are of particular interest. For instance, boilers and furnaces that use low quality fuels such as Bunker "A" and "C" grade fuels can typically accommodate volume fraction of 20 % -100 % blends of fish oil and therefore adding emusifiers to the fish oil or refining through transesterification or other biodiesel processing are not required; hence, cost of using biofuel is significantly reduced [9]. Other advantages of using fish oil include CO_2 or GHG emission reduction, renewability, non-toxic fuel, and safe handling operations.

Operations involving fish processing generate considerable quantities of edible and inedible by-products. A percentage of the total catch of fish is discarded as processing leftovers such as heads, frames, trimmings, fins, skin and viscera. The bulk of which is considered residue and dumped, creating both disposal and pollution problems [11]. More complete utilization is achieved by conversion of leftovers into fishmeal and fish oils. Fishmeal plants produce fish oil as a major by-product. [6]. Fish oil recovered from fishmeal residue varies considerably (between a mass fraction of 1.4 % and 40.1 %) depending on the species, tissue [2] and season. By-product oil from fish processing and fishmeal plants could therefore be an important source of biofuel. Fish oil (edible and non-edible) can be recovered through several methods. Typically the recovery method selected attempts to minimize oil decomposition or denaturization of the products. The most common process employed in fish oil production is wet reduction, which enables

recovery of a high volume of fish oil and may require subsequent refining steps in order to make the fish oil edible [12]. Other conventional fish oil recovery processes include hydraulic pressing, vacuum distillation, urea crystallization, hexane/solvent extraction, and conventional crystallization. Each of these processes incorporate either high temperatures and/or the use of flammable or toxic solvents, which could result in loss of functional properties, denaturization of fish protein, and deterioration of oil quality and nutrition value (e.g. PUFA oxidation) [13-15]. In addition, the focus of these processes is typically recovery for edible, pharmaceutical or industrial application which must meet regulatory standards.

2.1 Extraction of oil from fish waste for fuel oil application

The fishmeal process is the most common source of fish oil, as oil is generated as a by-product in the fishmeal production. The fishmeal can be produced from fish caught specifically for fishmeal and fish oil such as menhaden, anchovy, capelin and sardines; incidental or by-catch from another fishery; and fish by-products regarded as off cuts from edible fisheries which include cuttings from filleting operations, fish cannery residue, roe fishery residue and, more recently, surimi processing by-product [16,17]. The nature and volume of residue from fish processing depends on the processing procedures, fish species and fish conditions, harvest season, nature of final products etc... [18]. Global fish production has been reported to be about 140 Mt with 63 Mt of fish processing by-product generated annually across the globe [19]. Pelagic fish form the core source of fishmeal and fish oil production and are also essential ingredients in fish feed used in

farming carnivorous species such as salmon and trout [17]. Other types of fish operations, such as fish filleting operations result in off cuts (offal) which are sourced from the skeletons, heads, trimmings, and viscera, and also lead to residue which can be up to 50 % of total mass of the raw material [17,20]. The solid by-product has approximately the same protein as the fish flesh [20]; meanwhile, the whole fish is the preference for meal and oil production due to high ash and phosphorus content of fish processing residue [17]. Tables 2.1 and 2.2 summarize the major fish raw materials involved in production of marine oils and their sources.

Table 2.1: Species of Fish Caught for Fish oil and fishmeal production

Species	Country
Anchovy	Peru, Chile, South Africa, Namibia, Mexico, Morocco
Jack (Horse) Mackerel	Peru, Chile, China, Vanuatu
Capelin	Norway, Iceland, Russian Federation
Menhaden	USA: Atlantic and Gulf of Mexico
Blue Whiting	Norway, UK, Russian Federation, Ireland
Sand eel	Denmark, Norway, Faroe Islands
Norway Pout	Denmark, Norway, Faroe Islands
Sprat	Denmark, Russian Federations

FAO statistics database [55] compiled and cited by Bimbo AP [16]

Table 2.2: Fish Trimmings (off-cuts) and other non-fish species used or could be used for fish oil and fishmeal production

Species	Country
Dogfish	Canada, USA
Salmon, Farmed	Canada, Norway, UK, Ireland, China, Faroe Islands, Australia
Salmon, Wild	Canada, USA - Alaska, Japan, Russian Federation
White Fish Spp.	Canada, USA - Alaska, UK, Chile
Catfish spp.	USA, Vietnam
Tuna Spp.	Thailand, Japan, USA, Australia, South Korea, China, France, Ecuador, Maldives Islands and many others
Pollock	USA-Alaska, Russia
Sardine/Pilchard	Peru, Chile, South Africa, Namibia, Japan, Spain, Mexico
Atlantic Herring	Canada, Iceland, Norway, Denmark, UK, Faroe Islands, Sweden, Ireland.
Mackerel Spp.	UK, Peru, Chile, South Africa, Ireland, Norway, Denmark, Spain, Namibia, Russian Federation, China, Thailand
Horse Mackerel	Angola, Mauritania, Morocco, Namibia, South Africa, Turkey, France, Ireland, Latvia, Lithuania, Netherlands, Norway, Russian Federation, Spain, Ukraine, New-Zealand
Hoki (Blue Grenadier)	Australia, New-Zealand

Non-Fish Species	
Krill	Norway, Poland, Ukraine, Japan, South Korea
Squid	Argentina, Chile, Peru, USA, Japan, China, South Korea, Russian Federation, France, Portugal, Spain, UK, Morocco, Mexico, Hong Kong, Taiwan, Ghana, Mauritania, South Africa, Senegal, Tunisia, Falkaland Islands, Indonesia, Malaysia, Philippines, Thailand, New-Zealand.
Single-Cell Organisms	USA, Japan, Australia, Canada, USA (Hawaii), Israel, India

FAO statistics database [55] compiled and cited by Bimbo AP [16]

2.2 Fishmeal Operations for meal and oil productions

Fishmeal and fish oil are very broad generic terms often used for aquatic animal products derived through processing whole fish and/or fish/shellfish by-product [21]. Fishmeal plants for commercial production of edible fats and oils vary according to the type of raw materials. The principal fishmeal and oil processing is typically wet reduction or wet pressing [16,17]. The process starts with cooking to coagulate the fish protein and release bound water and oil, followed by pressing to squeeze out liquids (press water) from solids (press cake). The press water is desludged to further remove any solid particles followed by centrifugation of the liquid (press water) to separate oil from water [16,17]. There are also several processes that can be used to convert whole fish and/or fish residue into fishmeal and oil; they include wet rendering, hydrolysis, silage production (also

called autolysis), dry rendering and solvent extraction [16]. Figure 2.1 outlines the basic stages of fishmeal and oil production in a large scale fishmeal plant.

2.2.1 Oil Extraction Processes.

Fish oil extraction processes can be classified into three categories: physical, biological and chemical. Physical extraction processes include homogenizing, heating, pressing and filtering [6], also regarded as wet rendering [16]. The majority of the fish oil producing factories worldwide employs the wet rendering process as outlined in Figure 2.1. Biological processes include enzymatic oil extractions and silage production through the use of enzymes from fish viscera residue (autolysis) or enzymes from other sources (hydrolysis) [16]. Chemical solvent extraction is another well-established process to extract fish oil using organic solvents, however, the use of toxic solvents results in protein denaturization and loss of functional properties [15,22]. Supercritical fluid extraction technology (SCFE) has also been proposed in the extraction of compounds from natural sources [23] including oil recovery from seeds/biomass, raw fish and/or fish by-products [24]. SC- CO_2 for oil recovery is an attractive option as it is a non-toxic, non-flammable, inexpensive and clean solvent [15,25].

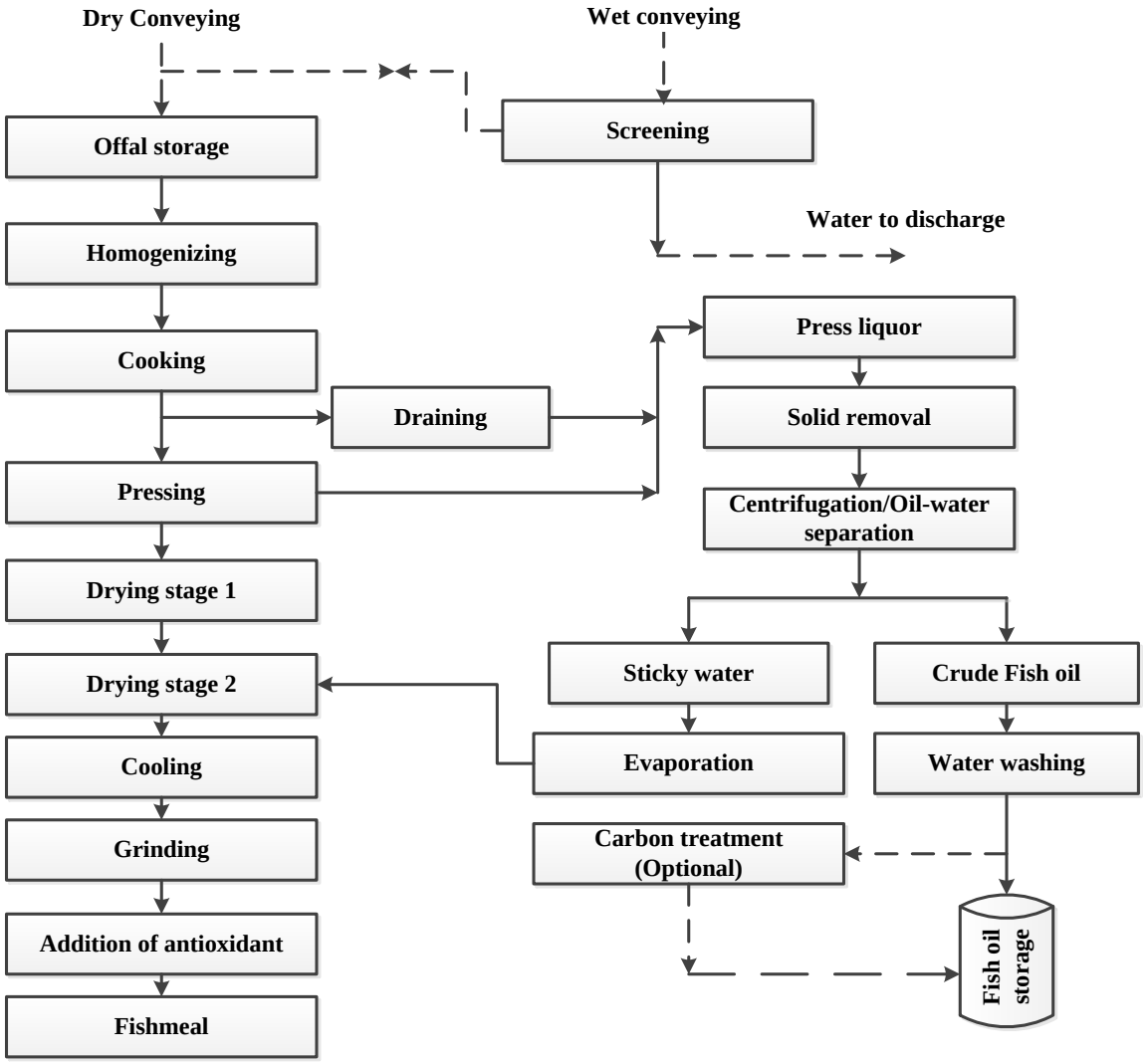

Figure 2.1: Basic Stages of Fishmeal and Fish oil Production at a Large -Scale Fishmeal Plant. Modified from Bimbo AP [16]

2.2.2 Physical Method

2.2.2.1 Oil Production from Fishmeal Process

The basic processing steps involved in the extraction of marine oils include cooking/heating or rendering, pressing, drying and milling. The cooking step is designed to rupture the fat cells for release of oils and pressing or centrifugation to separate liquids

(water, soluble protein and oil) from mass (solid cake) [6,21,26-28]. The raw materials, referred to as "offal", are gradually collected in pits/tank until there is a sufficient quantity for the fishmeal operation. The offal with large pieces is crushed (hashed) by machine prior to cooking [29]. Cooking coagulates the protein, ruptures the fat deposits and liberates oil and physico-chemically bound water. The cooking step also prepares materials for subsequent operations in various processing units. Most conventional heating methods use a steam cooker with a heated rotary screw, which conveys materials for continuous heating operation. Fish protein coagulation occurs at about 75 ℃ but cooking is typically in the temperature range of 95 ℃ - 100 ℃ for duration of (15 - 20) minutes [28]. Direct and indirect cookers (steam heated jacket, e.g. jacketed kettle) are also used.

The pressing operation removes as much liquid ("press liquor") as possible from the solid ("press cake"). The press cake is dried and milled to produce fishmeal. The pressing stage is also important for oil yield improvement, and chemicals such as formaldehyde or calcium chloride are sometimes used in pressing operation. However the addition can either reduce the oil nutrition value and/or increase residual chloride in the meal [6,26,28]. Press liquor consists of water, oil and solid particles. Oil content is in direct proportion to lipid content of the raw materials and separation of the three different fractions is accomplished through centrifuges. Separation efficiency depends on both centrifuge design and its mode of operation. Adequate temperature control is also required for effective centrifugation (about 60 ℃). The fishmeal and fish oil continues in separate lines after this process step.

Edible oils for human consumption require a refining/polishing step, which may include degumming, alkali refining, bleaching and deodorization as well as antioxidant addition. Impurities and other degraded/denaturized materials such as protein, suspended mucilaginous and colloid-like matters, oxidation products of fatty acids, vitamins, sterols, hydrocarbons, pigments, phospholipids, mono- and di-acylglycerols, and free fatty acids are removed from the oil. Polishing is also usually facilitated using hot water to extract impurities from the oils to ensure stability during storage. Marine oils processing steps are basically similar to those of vegetable oils; however, vegetable oil is more uniform with respect to composition than that of marine oils [26,28].

2.2.3 Chemical Methods

2.2.3.1 Solvent Extraction

Solvent extraction is the traditional lipid extraction method. Oils are highly soluble in organic solvents such as hexane, benzene, cyclohexane, acetone and chloroform. Organic solvents rupture plant cell walls [30] or disrupt the interaction forces between lipids and tissue matrix [31] and extract the oil. Factors to be considered in choosing an organic solvent include preferential solubility of the compound of interest, low boiling point for easy recovery, economics, toxicity, availability, and re-usability. Hexane is one of the few solvents with such qualities and used in large scale extraction [30]. There are various forms of lipids in tissue matrix. The simple lipids exist as part of large aggregates in storage and are readily extractable. The complex lipids usually exist as a constituent of membranes in close association with protein and polysaccharides therefore, due to interaction with these compounds; it is not extracted as easily [31].

Lipid solubility depends on the relative strength of interactions between the solvent and either the hydrophobic or hydrophilic constituents of the molecules. Low polarity lipid groups such as triacylglycerol (TAG) or cholesterol esters are readily soluble in organic solvents such as hexane, cyclohexane or toluene and higher polarity solvents like chloroform or ethers. In contrast, TAG and cholesterol esters tend to be insoluble in polar solvents such as alcohols (particularly methanol) [31]. Efficient lipid extraction requires total solvent penetration into lipid storage and matching polarity of the targeted compounds. It is therefore imperative that the solvents make physical contact with lipids and as such, mechanical disruption of the cell is required prior to solvent addition through grinding/homogenization in case of animal tissue.

2.2.3.2 Bligh and Dyer (B&D)

The traditional Bligh and Dyer (B&D) method is considered one of the best for polar lipid extraction from fish tissue and serves as a benchmark for comparison between other solvent extraction methods [11,30,31]. The B&D method is an adaptation (in terms of solvent volumes) of the Folch procedures [31] and procedures for optimum lipid extraction as reported in the literature [11,31]. The B&D method uses polar and non-polar solvents (e.g. chloroform, methanol and water) mixtures in specific ratios for total lipid extraction from muscles such as fish tissue. Norziah et al. [11] reported that the mixture ratios of chloroform/methanol/water of 4:2:1 and 2:4:1 gave higher total lipid yield. In a study by Tanamati et al. [32] nine methods were used to extract fat from ground beef and the authors determined that B&D methods provided the best yields. The solvents are required to penetrate into the fat cells and extract the lipid from the cell

membrane and muscles including the phospholipids materials [11,31]. Drawbacks of this method include the generation of large quantities of waste solvents in case of large scale application, making solvent recycling costly and raising safety concerns about handling organic solvents. Product contamination with the organic solvent has limited this application in food processing [30] and EU regulations in the use of chlorinated solvent have increased the cost of solvent disposal. As such, attempts have been made to modify the B&D method using non-chlorinated solvents but this has proved to be less successful compared to B&D method which is still widely used [31]. Table 2.3 illustrates different modifications to B&D method for lipid extraction.

Table 2.3: Total, Neutral, and Polar Lipids from High-Fat Beef using different solvent extraction method (g kg^{-1} fresh weight of beef) modified from Tanamati et al. [32]

Method	Solvents	Total Lipids	TAG	Other	Polar lipids
Folch et al. [56]	Chloroform/methanol	208±9	186.8±0.4	15.3±0.4	5.3±0.3
Bligh and Dyer [57]	Chloroform/methanol	201±4	181.7±9	13.9±0.3	5.2±0.3
Undeland et al. [58]	Chloroform/methanol	197.3±0.2	180±0.3	12.2±0.2	5.1±0.2
Smedes [59]	Cyclohexane/propan-2-ol	184±5	167.3±0.2	12.2±0.3	4.2±0.2
Hara and Radin [60]	n-Hexane/ propan-2-ol	185.4±0.5	169.3±0.3	11.4±0.4	4.6±0.2
Croon and Wallim [61]	Hydrochloric acid/diethylether/ petroleum-ether	185±3	166.8±0.3	13.4±0.4	4.8±0.2
Cunniff [62]	Ammonia/methanol/ diethylether/ petroleum ether	182.2±0.2	165.1±0.2	12.8±0.1	4.2±0.3
Burton et al. [63]	n-Hexane/ethanol/n-heptane	165.4±0.2	151.0±0.2	10.8±0.1	3.6±0.3
Cunniff [62]	Petroleum ether	161±7	151.4±0.2	7.1±0.2	2.4±0.2

2.2.3.3 Soxhlet Method

The principle is based on solid - liquid extraction (leaching), and has been the standard method for over a century [33]. Non polar solvents such as hexane, ethyl acetate or petroleum ether are used for lipid extraction. Extraction is through repeated washing or percolation of fresh organic solvent under reflux from a distillation flask. Extraction efficiency for different compound classes is highly dependent on the properties of organic solvent. This method under normal circumstances effectively determines TAG content but the extraction of phospholipids is incomplete. The advantages of the conventional soxhlet method include: sample phase is repeatedly brought into contact with fresh solvent ensuring complete extraction; heat applied to distillation flask is extended to the extraction cavity to some extent, hence, keeping the system's temperature relatively high, filtration of the extract is not required after extraction; and sample throughput can be increased by performing several parallel extractions simultaneously [31,33,34]. However, drawbacks include time required for extraction, environmental concerns over the generation of large amounts of organic wastes, which are costly to dispose of, and this method is not readily automated [31,33,34].

Furthermore, the method is appropriate for small scale extractions and has not been used in commercial or industrial scale application. Modifications to this method have focused on shortening the extraction time, the use of auxiliary forms of energy and automating the extraction apparatus [33] and include: high pressure soxhlet extraction, microwave-assited soxhlet extraction, ultrasound-assisted soxhlet extraction, automated soxhlet extraction, and focused microwave-assisted soxhlet extraction [33,34]. Automated and

microwave assisted soxhlet extraction has been applied commercially to environmental and food applications [33]. Rubio-Rodriguez et al. [25] compares soxhlet extraction to supercritical CO_2 extraction of lipids (Table 2.4). The overall total fatty acid extracted by supercritical CO_2 extraction is higher than that extracted by soxhlet extraction method, and since large scale application of this process (soxhlet extraction) for high volume of oil recovery is limited, supercritical CO_2 extraction may be a feasible alternative for biofuel production.

2.2.3.4 Accelerated Solvent Extraction (ASE)

Many organic solvents used for extraction boil at a relatively low temperature. This limits soxhlet extraction or automated soxhlet extraction, as the maximum temperature would be the solvent's boiling point. At higher pressures, the higher temperature can be achieved without boiling off solvent [30]. Accelerated Solvent Extraction (ASE) does not require the manual steps involved in preparing samples for analysis, has an increased reproducibility, and accelerates the process significantly. ASE was developed for the extraction of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) from solids [35]. Compared to other methods, there are fewer reported studies. However, in the last decade, ASE was used in lipid isolation from plant and animal tissue, egg-containing foods and dairy products [36]. Dodds et al. [36] also demonstrated the application of ASE for lipid extraction from fish tissue on a small scale using various organic solvents for extraction. Although, these studies have established ASE as a viable lipid recovery method, research and development in large scale recovery and feasibility is still required. High volume lipid recovery has been rarely reported with this process and

therefore, oil quality for biofuel application remains to be pursued. Also, environmental concern as per high volume of residual solvent will most likely be among the major constraints.

2.2.3.5 Microwave assisted extraction (MAE)

Microwave assisted extraction (MAE) utilizes microwave energy to heat solvents in contact with the solid matrix to extract compounds of interest from sample into solvent. Literature on MAE applied to lipid extractions are limited, but indicate improved lipid yields and reproducibility; however, this process is still required to be tested on a wide range of sample matrices [37]. MAE is considered superior to traditional solvent extraction with advantages of lower temperature, higher extraction rates, automation, and possibility of simultaneously extracting different types of samples [38].

MAE is based on the principle that the microwave heating system is very selective, and loses very little heat to the surroundings. The direct heating affects polar solvents and/or materials, so when used on samples, the moisture content of cells is reduced. This results in the generation of a significant amount of pressure which ruptures tissue cell membranes (animal cell) or walls (plant cell) releasing the cellular material [30]. A laboratory scale study by Ramalhsa et al. [37] for fish fat extraction extracted total lipid content similar to or higher than that of traditional extraction methods with reproducibility.

Ultrasonic-assisted solvent extraction has also been used for accelerated extraction of phenolic compounds from plant matrices. This process is considered effective compared to other conventional extraction methods as it allows cell disruption to permit phenolic

molecule interactions with solvents at a reasonably low temperature [39]. Since the microwave heat energy directly affects and reduces the polar and/or water content of fat cells, the overall effect on oil recovery might be a lower water content which would favor biofuel applications.

2.2.3.6 Acid-Alkali-aided Extraction

Fish protein isolate and fish oil are produced when using alkali or acid to digest the muscle protein. This method was suggested as a replacement for the surimi process (fish-paste making process) but it is now used for protein recovery from fish by-products. If the oil content of the fish residue is high, fish oil would also be produced [16]. The acid digestion method involves hydrolysis of muscle sample to release complex and/or bound fatty materials, thereby permitting total fat extraction using appropriate solvents [31]. As described by Xiao [31], the sample is hydrolyzed, fat is extracted using diethyl and petroleum ether solvents, the solvent is evaporated off, followed by the methylation of extracted fats. The drawback of this method is that non-lipid compounds are extracted along with lipids, leading to over-estimation of total fat in food samples. Acid hydrolysis is also considered an extremely aggressive method, which produce chemically degraded extracts unsuitable for fatty acid profiling [31]. This method might not be suitable for oil extraction for biofuel purposes as non-lipid compounds are extracted along with lipids. The implication on biofuel applications might be poor flow and combustion properties coupled with an increased knocking tendency while used in combustion engines.

2.2.3.7 Supercritical Fluid Extraction (SCFE)

Supercritical fluid extraction (SCFE) has been used to extract high-value products from plant tissue (e.g. micro algae) [13,30], and from animal tissue (e.g. fish by-products) [12,14,15,25,40]. SCFE also offers the benefits of being free of potentially toxic solvents, faster extraction and separation, and a safe/low thermal process for thermal sensitive products [30]. At supercritical conditions, fluids have gas-like viscosities and liquid-like densities, enhancing the solvation and flow properties [30]. SCFE also has the advantage of process flexibility due to the ability to modify solvent power or selectivity of the supercritical fluid. Several compounds have been studied for SCFE application such as carbon dioxide, hexane, pentane, butane, nitrous oxide, sulphur-hexafloride and florinated hydrocarbons [23].

Carbon dioxide (CO_2) is the most popular SCFE solvent as it is: readily available at low cost, low toxicity, non-flammable, high diffusivity with tune-able solvent power, and offers a potential use of greenhouse gas. In addition, CO_2 is a gas at ambient conditions making it very simple to separate solvent from the solute. CO_2 also has mild critical conditions (T_c = 303.9 K, P_c = 7.38 MPa) relative to other solvents [12,13,23-25,41].

Four major factors controlling supercritical CO_2 extraction (SC-CO_2) are pressure, temperature, CO_2 flow rate, and extraction time [30]. Esquivel et al. [42] extracted Sardine oil at 12.6 MPa and 18 MPa, and 313 K and 321 K using SC-CO_2. The rate of sardine extraction was sensitive to temperature and pressure, and the sardine oil loading in CO_2 increased with increasing pressure and decreased with increasing temperature.

In general, the main limitation of supercritical CO_2 extraction is its low polarity. Supercritical CO_2 is a good solvent for non-polar (lipophilic) compounds; hence, it has low affinity for polar compounds. Heavy metal extraction along with oil is also reportedly almost impossible [12]. This property is beneficial for using oil product in biofuel application. In order to extend the solvent polarity, employing polar modifiers (co-solvents) to change the solvent polarity and to increase its solvation power towards the compound of interest has been suggested. The addition of a relatively small percentage of alcohol, e.g. (mass fraction of 1 % - 10 %) methanol to carbon dioxide, has reportedly expanded the solvents extraction range to include more polar analytes [14,23,24]. The addition of ethanol to supercritical CO_2 (mass fraction of 10 % - 15 %) has also been reported by Mercer and Armenta [30] to increase its polarity and to change its viscosity. The resulting effect is an increase in solvating power of CO_2, and the extraction requires low temperature and pressure. Catchpole et al. [43] determined that fish oil solubility increased exponentially with the addition of a mass fraction of 12 % of ethanol to CO_2 leading to better recovery as compared to pure CO_2 at the same temperature and pressure. In this regard, more polar lipids would have been extracted along with the oil.

In the context of using the raw extracted oil as biofuel or blend of oil, the presence of polar lipids such as phospholipids and glycolipids could decrease the oil quality and/or flow properties, hence, addition of co-solvents may not be desired. In addition, the co-solvent is liquid at room temperature and atmospheric pressure and would be extracted

along with compound of interest; therefore, subsequent processing for solvent removal will be required [23].

Supercritical CO_2 extraction is sensitive to the water bound in the sample matrix. The water in a sample matrix reduces contact time between solvent and solute. The moisture acts as a barrier against CO_2 diffusion into the sample, and the diffusion of lipids out of the cells, hence, freeze drying of the sample is required prior to SCFE [30]. Dunford et al. [14] investigated the effect of water presence in the sample on extraction of Atlantic mackerel oil using supercritical CO_2. Feedstock with a mass fraction of 10.2 % water content treated with SC-CO_2 resulted in higher oil yields (2.7 g) compared to those with a mass fraction of 26.0 % and 64.0 % water content with 2.5 g and 0.3 g yields respectively. Given that fish processing by-products will contain high water levels this will impact the recovery of the oil and therefore pretreatment process may be required.

SCFE has been widely used in food and natural products extraction, for process development, and to extract targeted (bioactive or valuable) compounds from different matrices [12,14,15,24,25]. There is little data on the feasibility of the industrial scale application of the SCFE process. Sahena et al. [15] (Table 3) compares fatty acid profiles of fish oil extracted using SC-CO_2 with different modifications and soxhlet extraction. The variation of fatty acid profiles with regards to different extraction process can be deduced therein. Due to the high selectivity SC-CO_2 extraction preferentially extracting more saturated fatty acids (a mass fraction of 21.09 %) compared to the modified SC-CO_2 and soxhlet extraction processes (extracted a mass fraction of between 18.20 % and

18.87 %). In contrast, the modified SC-CO_2 and soxhlet extraction processes extract more polyunsaturated fatty acids (a mass fraction of between 59.59 % and 60.53 %) than the SC-CO_2 extraction (extracted a mass fraction of 56.32 %). High content of polyunsaturated fatty acid species could be advantageous to thermal and cold flow properties of fuel oil, as oil with a high degree of unsaturated fatty acid has a low melting point compared to oil with higher degree of saturated fatty acid which melts at a higher temperature [44,45]. The disadvantage of having more polyunsaturated fatty acids in fuel oils is the increased rate of oxidation when compared to the saturated species**.** Lin and Li [46] reported that biodiesel with polyunsaturated fatty acids with double bonds of more than three are susceptible to deterioration, thus causing precipitation in the combustion chamber of the combustion engine.

SC-CO_2 is highly selective towards non-polar compounds, therefore, heavy metal extraction (e.g Cd, Hg, Pb and As) along with fish oil will be negligible compared to other processes. This fact is corroborated by Rubio-Rodriguez et al. [12]. Table 2.4 summarizes the advantages and disadvantages of various solvent extraction methods. Selection of any of the processes will be based on cost consideration, environmental impact, and analyte selectivity.

Table 2.4: Comparison between solvent extraction processes, merits and demerits

Method	Solvents	Advantages	Disadvantages
Bligh and Dyer	Methanol, chloroform, and water	Simple and standard method. Well established. Determines total lipids. Direct analysis of samples with no pre-drying.	Adverse effects of chloroform on the environment (EU regulation controlling chlorinated solvents). Laborious (requires filtration etc…)
Soxhlet method	Organic solvents (e.g. hexane, toluene, acetone, petroleum ether, cyclohexane)	It's simple. Not very labor intensive. Can be operated with non-chlorinated solvents. Lipids can be further used.	Lower yields than Bligh & Dyer method. Extractable lipids are determined not total lipids. Large amount of solvents needed. Special equipment required. Results are very much operationally dependent (Solvent composition, extraction time cycles). Conditions are difficult to control (Continuous flow of solvents). Time consuming.
Accelerated solvent extraction (ASE)	Dichloromethane /hexane,	Not very labor intensive. Lipids can be further used. Method takes out environmental contaminants (e.g. PCBs, dioxins, pesticides).	Expensive. Not all lipids extracted. Various mixtures of solvents. High temperature and pressures required. Drying of samples required.
Supercritical fluid extraction (SFE)	CO_2	Rapid. Requires no organic solvent Lipids can be used for further analysis.	Very expensive equipment. Complex equipment. Supply of CO_2 needed.

2.2.3.8 Membrane coupled SC-CO_2 extraction.

Sarrade et al. [47] attempted coupling an SC-CO_2 extraction process with a membrane system (nanofiltration) for separation of CO_2 from the resulting mixture. In 1998, the authors [48] coupled nanofiltration tubular membranes resistant enough to endure the supercritical conditions. The goal of coupled process is to extract triglycerides directly from fish oil. The process further separated the product into two parts: the short-chained (lowest molecular weight) triglycerides as permeate and the long chained (heaviest molecular weight) triglycerides as the retentate i.e. EPA and DHA. A modification of this process was proposed in 2002 to enhance the extraction process in lowering the energy required for CO_2 recycling and to purify the low molecular weight compounds [49]. Considering the fact that resulting products from this innovation are of high level of purity and separation, its application would be required in areas where high purity products and high level of separation is required, such as food processing, biotechnology, cosmetic and pharmaceutical industries. Hence, for the purpose of biofuel application where fractionation of triglycerides is not required, this process might not be useful.

2.2.4 Biological Processes

2.2.4.1 Hydrolysis

Fish protein concentrate is produced in the fish processing industry using enzymatic hydrolysis (Figure 2.2). Silage is the autolysate or fish digest recovered using internal enzymes (enzymes from fish viscera) and acid for stability. This process is referred to as autolysis. If exogenous enzymes are used (enzymes from other sources) the process is

called hydrolysis. Acid addition inhibits and destroys the bacteria enabling internal (fish) enzymes to digest the fish muscle [16,50]. Hydrolysis can also be carried out chemically under alkaline or acidic conditions. Raw materials with low lipid content result in a process where no oil is produced as by-products [50], whereas, lipid-protein emulsions can be formed with high lipid content feedstock and thus reduce the oil yield [6] and quality. Generally, autolysis of fish muscle is usually performed to produce fish silage. The process forms an aqueous solution rich in small peptides and amino acids, and the enzymes promote the release of oil [22]. The disadvantage of autolysis is time consumption, which reduces hydrolysates quality and it is difficult to control the process since internal digestive enzymes are required [6,22,51].

The use of exogenous enzymes (enzymes from other sources) makes hydrolysis process highly controllable with reduced residence time [22,51]. Enzymes can be sourced from either animal, vegetable or microbial to accelerate the breakdown of proteins into smaller units (peptides) [16,52]. Enzymatic hydrolysis using commercial low cost and food grade protease provides an attractive alternative as the process is carried out under mild conditions for short period of time. Commercial protease has been used to produce oil from marine by-products, resulting in improved yields as compared to yields achieved from cooking processes [4]. However, product deteriorates due to favorable conditions for hydrolysis of free fatty acid (FFA) and oil-water emulsion resulting in high FFA content which might not be suitable for biofuel application.

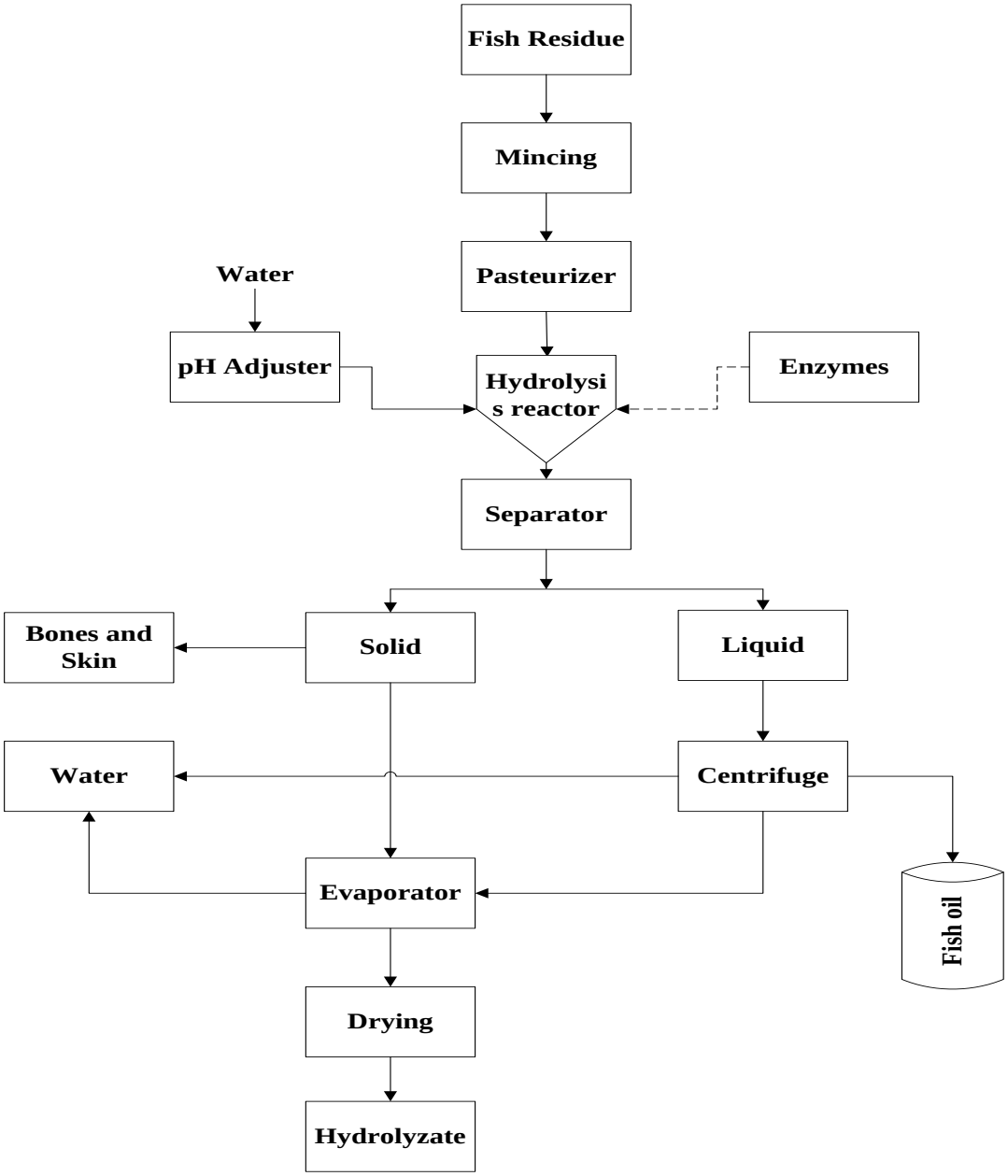

Figure 2.2: Flow process for typical hydrolysis. Modified from Bimbo AP [16]

2.2.4.2 Fermentation

Fermentation is a biological process which produces silage and oil when minced fish material is mixed with a carbohydrate sources (e.g. sugar or molasses) or organic acids

(e.g. lactic acid) and bacteria [6,50]. Biological fish ensilage has been shown to have a good nutritional value relative to acid fish silage [53]. Biological ensilage is preferable; especially in tropical regions due to adequate supply of carbohydrate sources and low cost [53]. The success of the fermentation process depends on lactic acid generated by micro-organisms, which must be sufficient enough to lower the process pH to around 4.5 [6,53], and remain stable during storage to prevent growth of bacteria [53]. The lipid oxidation of oil produced during fermentation was studied by de Lurdes et al. [53] and it was determined that oils obtained from silage with added formaldehydes had a substantially higher peroxide value than oils from biological silage. Figure 2.3 is an outline of a typical biological fermentation process. Oil released from the fish materials during fermentation is separated by centrifugation [6]. Oil with high levels of polyunsaturated fatty acids (PUFA) makes the silage prone to oxidation, rendering the feed unpalatable and/or unsafe for livestock. Fermentation using lactic acid has beneficial effects on lipids in fish silage as it stabilizes the oil and improves quality for animal feed [53] Fermentation is a promising recovery process due to its possible energy saving, low cost and recovery of better nutritional value products.

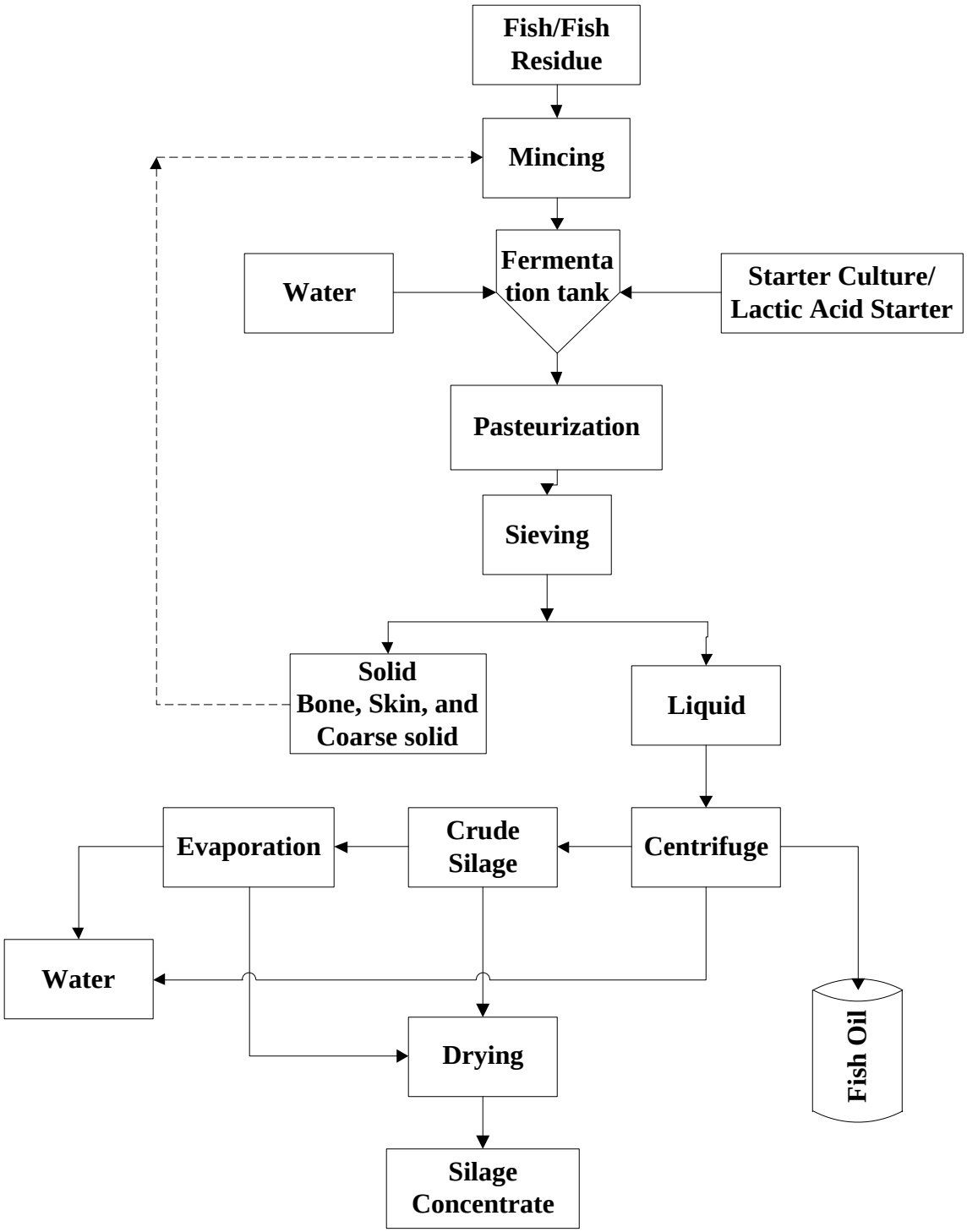

Figure 2.3: Flow process for typical fermentation. Modified from Bimbo AP [16]

2.3.0 Summary and conclusions.

The recovery of oil from fish by-product for use as a fuel can be a sustainable cost effective strategy for conventional combustors, boiler engines, or in-house use. The oil product does not need to meet high purity standards associated with edible oils but must balance fuel quality with costs, process complexity and be robust enough to handle different types and quality of fish residue. This review focuses on various extraction processes for lipid/oil from fish residues which could be used for bio-fuel application.

The fishmeal and physical processes are typically used to produce fishmeal with crude fish oil as a by-product. The quality of oil by-product from these processes can be good enough for biofuel application, for instance, fuel oil for convectional combustors or diesel engines that use low quality fuels (e.g boilers and furnaces). The oil may also require subsequent refining steps in order to make it edible. However, the intensity of the process with respect to temperature (prolonged or repeated heating or cooking period) can degrade oil quality. High energy input required for cooking, pressing and centrifugation may lead to high production costs for this process.

The chemical processes result in high yields of total lipid. However, solvent extraction systems may also extract non lipid material which can negatively impact oil quality. The commercial scale application of these processes can be problematic due to large volumes of residual solvent, and degraded product quality due to toxic solvent. The majority of solvent extraction processes (for example B&D, Soxhlet, MAE, and ASE processes) were

developed for lipid analysis and therefore impractical for extraction of fuel grade quality oils.

SC-CO_2, although limited in large scale application, can reduce the environmental impacts and safety issues involved in conventional solvent extraction processes. Theoretically, it can achieve a high quality product recovery as the process would produce a high quality fuel oil with minimal impurities; however, the viability of large scale production is limited. The costs and infrastructure associated with the required high pressures and pre-treatment (drying) have not been assessed. The quality of oil recovered by SC-CO_2 for use as a stand-alone low grade fuel oil and/or blends of bio-fuel for combustion engine applications is an area requiring further investigation.

Biological processes are used on a larger scale for protein concentrate and can lead to high volume oil production. The processes are environmentally friendly and safe; they require low temperature and/or low or reduced energy input. However the product deteriorate due to long residence times, and favorable conditions for hydrolysis of FFA and oil-water emulsion therefore further downstream processing is also required. Costly enzymes and limited process control are among other factors that hinders its application.

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CHAPTER 3

THERMAL AND FLOW PROPERTIES OF FISH OIL BLENDS WITH BUNKER FUEL OIL

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Abstract

The thermal, flow, and heating properties of unrefined fish oils (anchovy-sardine oil derived from fish processing waste, and unrefined salmon oil derived from salmon discards) blended with bunker 'A' oil (a common heating oil) were investigated. The rheological properties of the blends were examined and modeled using an Arrhenius equation approach. The onset of thermal degradation of waste fish oil, salmon oil and bunker 'A' oil were 187 ℃, 229 ℃, and 75 ℃ respectively and complete decomposition of the oils occurred between 500 and 550 ℃. The flow behavior index of the oil/blend samples was less than one, which indicated that the fish oil/blend exhibited non-Newtonian fluid behavior. More so, all samples showed decreasing viscosity with increasing shear rate indicating that the samples and their blends exhibited a shear-thinning non-Newtonian behavior. The average heating value of anchovy-sardine oil, unrefined salmon oil and bunker fuel oil were 38.69, 39.51 and 43.36 $MJkg^{-1}$ respectively. The energy barrier to flow and viscosity of the blends decreased with increasing quantity of fish oils in fish-bunker mixture.

This paper has been published in the *Fuel journal*. Ibraheem Adeoti is the lead author and the co-authors are Marina Santos and Dr. Kelly Hawboldt. Mr. Adeoti's contributions to this paper include the following:

- Wrote the paper
- Lead in performing all the laboratory testing and analyses (except where noted)

- Conducted all data processing and interpretation of results
- Performed all literature searches required for background information

Ms. Marina Santos helped during laboratory experiment and testing such as TGA analysis, DCS analysis, Extraction of fish oil, blending of the oil with petroleum fraction and flash point testing of the oil and blends. Dr. Hawboldt provided technical guidance and editing of the manuscript.

Nomenclature

A	frequency factor (Pa.s)
B100	100% biodiesel
CO	carbon monoxide emission
DHA	docosahexaenoic acid
DSC	differential scanning calorimeter
E_a	activation energy (kJ mol^{-1})
EPA	eicosapentaenoic acid
GHG	Greenhouse gas
HHV	higher heating values (MJ/kg)
K	consistency index (Pa.s^n)
k	reaction rate constant
MUFA	monounsaturated
n	flow behavior index (dimensionless unit)
OCN	ocean nutrition Canada
PUFA	polyunsaturated fatty acid
R	gas constant (8.314 J mol^{-1} K^{-1})
RFO	residual fuel oil (bunker fuel oil)
SB20	20% (v/v) unrefined salmon oil in 80% (v/v) bunker fuel oil
SB50	50% (v/v) unrefined salmon oil in 50% (v/v) bunker fuel oil
SB80	80% (v/v) unrefined salmon oil in 20% (v/v) bunker fuel oil

SFA	saturated fatty acid
SSE	sum of square errors
T	temperature (K)
TGA	thermogravimetric analysis
WB20	20% (v/v) waste fish oil in 80% (v/v) bunker fuel oil
WB50	50% (v/v) waste fish oil in 50% (v/v) bunker fuel oil
WB80	80% (v/v) waste fish oil in 20% (v/v) bunker fuel oil
ΔH	enthalpy (kJ/kg)
τ	Shear stress (Pa.s)
γ	Shear rate (s^{-1})
ω-3	omega -3

3.1 Introduction

Fish processing facilities generate a significant amount of fish by-products that could be source of energy, food, or industrial chemicals. While fish oil is a natural source of omega-3 polyunsaturated fatty acids (mostly eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) used in nutritional supplements, monetizing these oils may be challenging at processing facilities where there is limited infrastructure and plants are remotely located. Under these conditions, extraction of oil from fish by-product for use as an in-house or regional fuel may be both an economically and environmentally more sustainable approach. Processes to extract and refine fish oil for fuel are less energy intensive than those used for nutritional quality oil extraction/refining due to higher product quality standards required to meet nutritional regulations. Further, extraction of oil from waste (fish residue) for biofuels does not negatively impact food production [1].

Biofuels are gaining more attention due to availability, renewability and other advantages such as, net greenhouse gas (GHG) reduction, improved combustion, biodegradability and low toxicity [2]. Studies have proposed using fish oil and/or blending with petroleum based fuels as an alternative fuel oil for convectional combustors or diesel engines [3,4]. Fish oil has been used as fuel oil in power/heat generation as these systems can tolerate lower fuel quality than diesel engines [4]. Bunker fuel oils (No.2 to No.6) [5,6] are heavy petroleum distillates and are viscous, lower-grade fuel oil used to produce electricity, to fire boilers and blast furnaces in industry (notably the pulp and paper industry), and to power large marine and other vessels. Bunker fuels contain sulphur resulting in sulphur

dioxide as well as other contaminant emissions (due to the high molecular weight of the fuel) [7]. Blending fuel oils with unrefined fish oils could reduce emissions associated with this equipment particularly sulphur dioxide. An investigation by Preto et al., [4] reported that fish oil blends with No.6 and No.2 fuel oils burn well in conventional furnaces resulting in lower overall pollutant emission. The fish oil also reduced the viscosity of the fuels through blending [3]. While this work is promising, in order to use blends of fish oil and petroleum effectively, knowledge of thermal stability, rheological properties, enthalpy and other cold flow properties of the blends is required. There is limited literature on the properties of heavy oil-fish oil blends. A comparison of the quality of fish oil compared to the petroleum based fuels was done using thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC) among other methods [8-10]. Operations related to the mass transfer and liquid flow, such as pumping, storage and handling, are addressed with rheological knowledge of the fluid [8]. There are few published studies on the thermal stability and rheological properties of fish oil [8-10] and less on blends of fish oil and/waste fish oils with heavy petroleum oils. The objective of this work is to evaluate thermal stability, rheological, and heating value, of unrefined salmon oil, waste fish oil, and blends with bunker fuel oil.

3.2. Materials and Methods

3.2.1. Sample preparation

Fresh salmon waste (head, gut, trimming and frame) were obtained from the Centre for Aquaculture and Seafood Development (CASD), Memorial University of Newfoundland (MUN). The waste was stored at -40 ^{o}C. As part of the oil removal process, the waste was

thawed, ground to 1-4 mm and heated in a hot water bath at 80 ^{o}C for 10 to 15 minutes. The heated waste was transferred into eight 50mL centrifuge tubes and centrifuged in an Eppendorf centrifuge 5810 (Eppendorf AG 22331, Hamburg-Germany) at 3850rpm for 9 minutes. The fish oil was separated by decantation and stored again at -40 ℃. The same oil recovery method was used to recover oil from Atlantic cod, herring and mackerel. Bunker fuel oil and waste fish oil were supplied by St. Francis Xavier University, Nova Scotia. The waste fish oil is a by-product of Ocean Nutrition Canada Ltd. (ONC). Omega-3 fatty acids are extracted/concentrated from anchovy (95 – 99 %) and sardine (1-5 %) oil imported from Peru, off the coast of South America. The waste fish oil 'as is' was stored in the freezer at -40 ℃ until used while the bunker fuel oil kept in cold room at temperature between 0 and 4 ℃.

3.2.2. Thermogravimetric analysis (TGA)

The thermal stability of the unrefined salmon oil, and waste fish oil and their blends with the bunker fuel was conducted using the Thermo-gravimetric Analyzer (Model Q500, TA Instruments Inc.). Approximately 0.8 - 1.2 mg of oil/blend sample was loaded into the furnace. The TA instrument was manually programmed to heat up the sample from ambient temperature condition to 800℃ under N_2/air atmosphere at the ramping rate of 5 ℃/min. Sample weight change was automatically acquired every second and the data were analyzed and plotted using the TA Universal Analyzer Software.

3.2.3. Calorific value/High heating value (HHV)

The calorific value of a fuel is the thermal energy liberated per unit mass of fuel during a complete combustion reaction till the products of combustion are cooled back to the

initial temperature of the combustible materials [11]. Calorific value is a measure of the energy content in a fuel. The 1108 oxygen bomb calorimeter (Parr Instrument Company) was used to determine the HHV of the unrefined salmon and waste fish oils and the blends of each with bunker fuel oil according to ASTM D2015 standard method. Oxygen – bomb vessel was pressurized to approximately 3 MPa with an oxygen container. The bomb was ignited automatically after the jacket and a bucket temperature equilibrated to the desired temperature.

3.2.4 Flash point testing

The flash point of a fuel oil is the lowest temperature, corrected to atmospheric pressure, at which application of a naked/test flame cause the fuel-vapor to ignite under specific conditions of test [11]. The flash point of the oils and their blends were measured by Pensky-Martens closed cup apparatus (K16200 – Koehler Instrument Company Inc.). The flashpoints were determined in accordance with ASTM D93. The apparatus is equipped with closed cup to which the sample is fed and heated at a controlled rate. An ignition source was introduced and the temperature at which the heated oil flashes was recorded as the flash point.

3.2.5. Melting points and enthalpy

Melting points were determined in a METTLER TOLEDO DSC-1 (Differential Scanning Calorimeter) with Julabo intercooler and TA Universal Analyzer Software. Approximately 5-10 mg of sample was cooled to -60 ^{o}C and heating to 40 ^{o}C at 5 ^{o}C/min. The thermogram plots containing normalized heat flow with temperature were integrated to obtain peak onset and areas. The average melting points of unrefined salmon oil and

waste fish oil were analyzed and recorded. The thermograph peak was used to determine the enthalpy *(*Δ*H*) of the samples using the TA Universal Analyzer Software.

3.2.6. Rheological properties

The apparent viscosity of the fish oils and their blends with bunker fuel oil were measured using a Brookfield DV-III Ultra Programmable rheometer equipped with a small sample adapter and spindle (SC4-18/13RP). The temperature of the sample was kept constant by connecting a circulating water bath (NESLAB EX series) to a water jacket of the small sample adapter. Measurements were taken at 20, 30, 40, 50 and 60 ℃ in (Pa.s) by changing the spindle rotation speed from 10 rpm at intervals of 10 until the torque exceeded 100%. The power law (Eq.1) was used to analyze the flow behavior of all raw samples and their blends.

$$\tau = K\gamma^n \quad (1)$$

Where τ = shear stress (Pa.s), γ = shear rate (s^{-1}), K = consistency index ($Pa.s^n$) and n is the flow behavior index (dimensionless unit) [8,10,12]. Taking the logarithm of both sides of Eq.(1) yields a straight line plot of $\log \tau$ versus $\log \gamma$ with intercept ($\log K$) and slope n. The effect of temperature on apparent viscosity is outlined in Eq. (2) [8,10,13].

$$k = A \exp^{(-Ea/RT)} \quad (2)$$

Where k is the reaction rate constant, A is the frequency factor, E_a is the activation energy (J/mol), R is the gas constant (8.314 J/mol.K), and T is the temperature (K). The natural logarithm (ln) of apparent viscosity versus inverse of temperature (*1/T*) was plotted for each data set and with the slope of the straight line used to calculate E_a, and A as the intercept.

Statistical Analysis

The experiments were conducted in triplicates. Mean and standard deviation were calculated using Microsoft Excel 2007 (Window 8 Edition, Microsoft Corporation, USA). Analysis of variance (ANOVA) at alpha level of 0.05 was performed using the statistical tool in Microsoft Excel. Average of three measurements plus/minus standard deviation was given in tables where applicable.

3.3 Result and discusion

3.3.1 Thermal analysis (TGA)

Table 3.1 outlines the TGA results of raw fish oils and blends from this study and published studies. Included in this table are species data obtained in previous studies in our lab (herring and mackerel and Northern cod). The TGA analyzes the thermal stability by relating changes to its physicochemical properties to weight changes as a function of temperature [14]. The thermal behavior of bunker fuel oil, unrefined salmon oil and waste fish oil were studied under a nitrogen atmosphere between 0 °C and 800 °C (Fig. 3.1a). The weight of the bunker fuel oil starts to decrease from 50 °C – 100 °C which is likely due to vaporization of lighter fractions of the bunker fuel oil. The rate of weight loss is rapid between 100°C - 250 °C likely a result of decomposition of lighter components of the oil [8]. From 250 °C to approximately 350 °C, the weight loss rate decreases may be due impurities such as waxes, pigments and complex metallic compounds etc., which can be present in heavier fuel oil fractions [15]. The second rapid decomposition occurs between 375 °C and 450 °C. The oil was not completely decomposed at 500 °C and soot, ash, and other thermally recalcitrant substances

remained. The weight fraction of the soot/ash mixture was 12.3 wt. %. The onset weight loss temperature of waste fish oil and unrefined salmon oil starts at 187 °C and 229 °C respectively. Between 200 °C and 450 °C, the rate of weight loss of the waste fish oil increased rapidly with temperature (Fig. 3.1a). The total weight loss in this region is approximately 95 wt. %. Unrefined salmon oil did not decompose as rapidly in this range. Sathivel et al., Wiedermann and Chiou et al., [10,16,17], determined unprocessed salmon oil contains phospholipids, metals, minerals, free fatty acids, peroxides and their degraded products in the oil. These impurities absorb heat, slowing the decomposition of the oil [10,16] as such refined edible oils (waste fish oil) undergo more weight loss than unrefined edible oils [10]. In the temperature range from 350 °C-450 °C the salmon oil degrades rapidly (approximately 90 wt. %) and approximately 0.6 wt. % recalcitrant materials remained.

The waste fish oil exhibited higher volatility than the unrefined salmon oil. This is likely due to the removal of edible components of the oil (such as omega-3, omega-6, EPA, DHA etc…) by ONC prior to testing. The unrefined salmon oil composition is richer in long chain polyunsaturated fatty acids that are more susceptible to oxidation. Thermal polymerization reactions occur in edible oils in temperature range from 200 °C to 300 °C in inert or nitrogen environment [18,19], which was observed in the TG curve of unrefined salmon oil (Fig.3.1a). The average boiling point of edible oils ranges from 200 -250 °C depending on the type of oil as such weight loss at approximately 250 °C and below is considered vaporization.

Table 3.1: Data summary comparing decomposition temperature of the oils

	Decomposition Onset temperature °C		Rapid decomposition temperature range °C		Final decomposition temperature °C	
Oil Types	N_2	Air	N_2	Air	N_2	Air
Waste fish oil	187	187	200 - 450	200 - 450	464	537
Unrefined Salmon Oil	229	229	300 - 450	300 - 475	476	659
Herring	175		325 - 425		475	
Mackerel	75		275 - 425		475	
Northern Cod	250		350 - 450		475	
SB20[a]	58		125 - 450		475	
SB50[a]	100		175 - 450		475	
SB80[a]	125		325 - 450		475	
RFO[b]	50		100 - 420		450	
WB20[c]	75		150 - 450		450	
WB50[c]	75		150 - 450		450	
WB80[c]	100		200 - 450		450	
Salmon biodiesel [19]	140	–	214 - 217	–	360	–
Unrefined salmon oil [19]	220	–	403 - 440	–	480	–
Unrefined Pollock oil [12]	–	180	–	200 - 450	–	535
Unrefined Red Salmon oil [11]	–	180	–	200 - 450	–	533
Unrefined Pink Salmon oil [11]	–	210	–	200 - 450	–	668

[a] SB20, SB50, SB80 = 20, 50 and 80 % (v/v) unrefined salmon oil blend with RFO

[b] RFO = Residual fuel oil

[c] WB20, WB50, WB80 = 20, 50 and 80 % (v/v) unrefined salmon oil blend with RFO

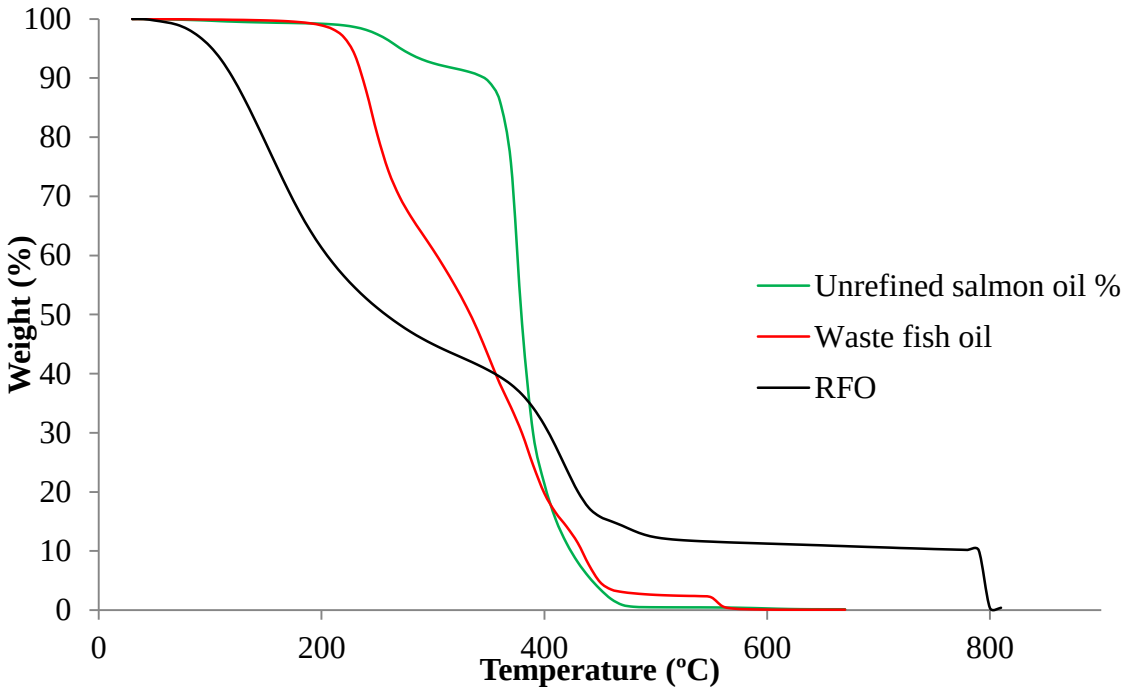

Figure 3.1a: Thermal degradation of the residual fuel oil, unrefined Salmon oil and waste fish oil under nitrogen and air atmosphere.

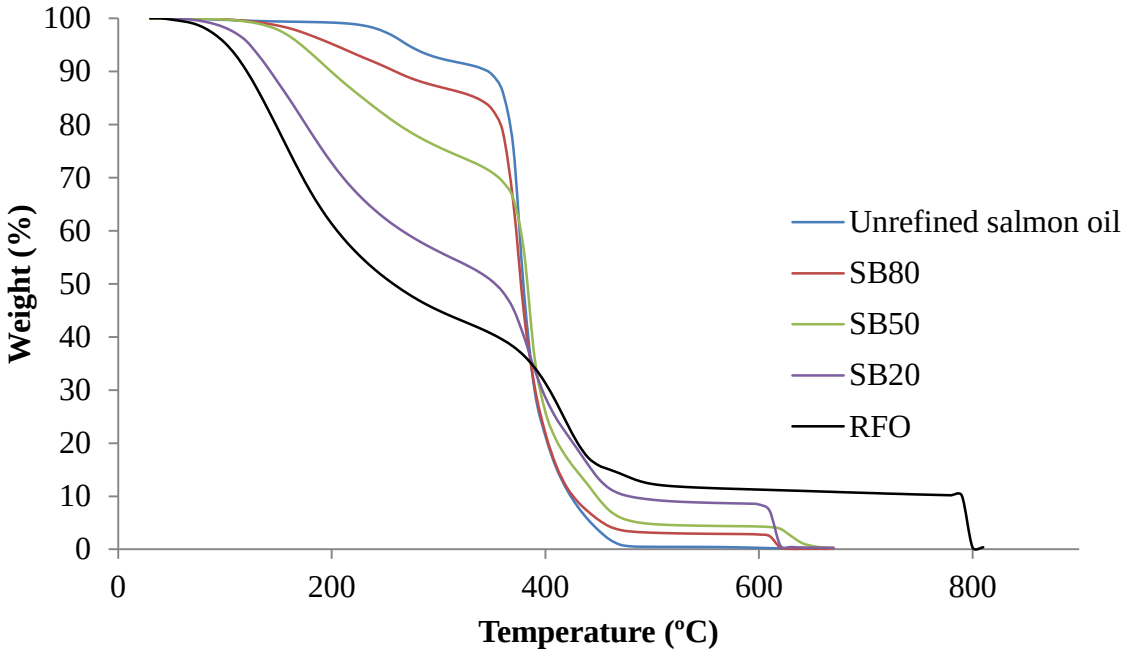

Figure 3.1b: Thermal degradation of blends SB20, SB50, SB80 of unrefined Salmon oil with residual fuel oil under nitrogen and air atmosphere.

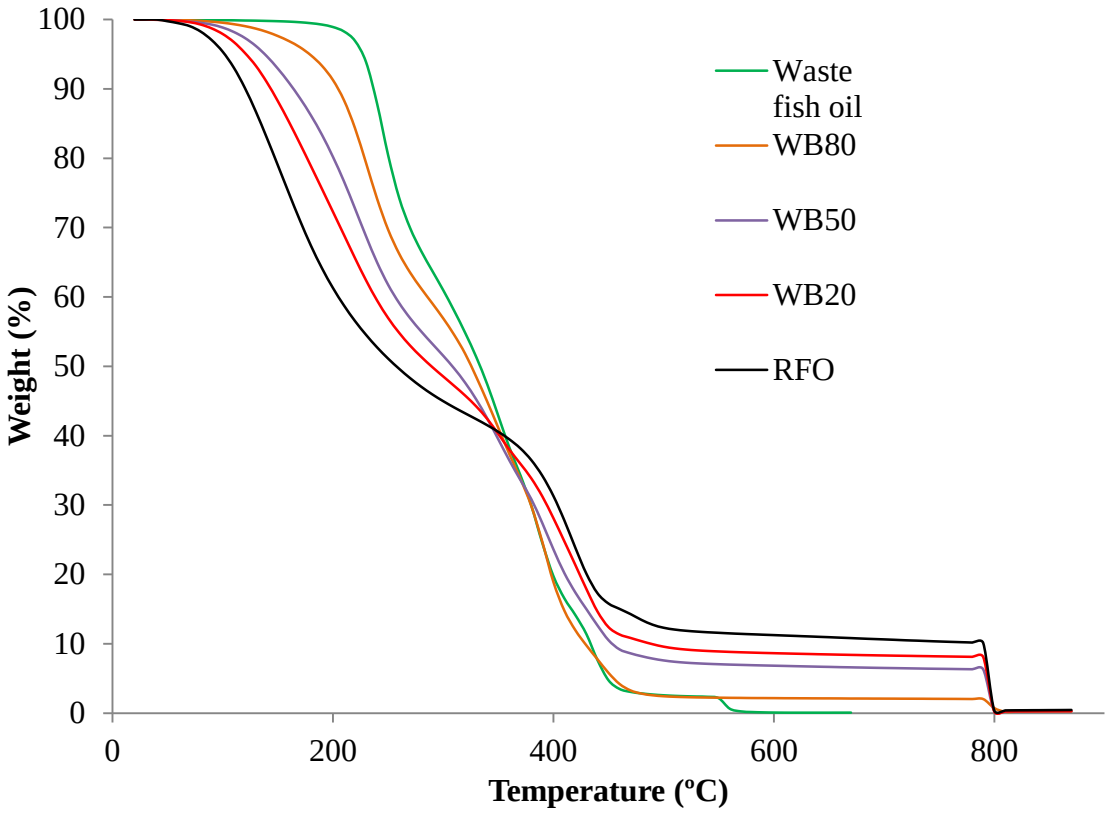

Figure 3.1c: Thermal degradation of blends WB20,WB50,WB80 of waste fish oil with residual fuel oil under nitrogen and air atmosphere.

The unrefined salmon oil blends blended with bunker fuel oil (Fig.3.1b) exhibit behavior similar to unrefined salmon oil. The deviation of TG-curve in each of SB20, SB50 and SB80 from unblended unrefined salmon oil corresponds to the proportion of RFO oil in the blends. The higher the RFO oil in the blend, the higher the percentage weight loss due to volatization and decomposition between temperature of 50 °C and 200 °C. Rapid thermal decomposition occurs for all the blends at temperatures between 350 °C - 450 °C. There is a crossover point at approximately 390 °C, where the effect of impurities in the fish oils may impact the rate of decomposition. The mass fraction of the undecomposed

portion (residue) decreases with increasing fish oil content; SB20, SB50 and SB80 oil blends at 475 °C were 10.08, 5.50 and 3.37 wt. % respectively.

Similar trends were observed with waste fish oil blends WB20, WB50 and WB80 (Fig. 3.1c). A steep slope was observed between 75 °C and 425 °C, representing a rapid decomposition, more than 90 wt. %. A crossover point is also observed at approximately 375 °C (Fig. 3.1c), where the effect of impurities in the fish oil again may be slowing thermal decomposition as discussed previously. The mass fraction of undecomposed residuals of WB20, WB50 and WB80 oil blends at 475 °C were 9.28, 6.99 and 3.14 wt. % respectively.

3.3.2 Calorific value/High heating value (HHV)

The higher heating values (HHVs) and flash points are outlined in Table 3.2. The HHV of the waste fish oil and unrefined salmon oil are 38.69 MJ/kg and 39.51MJ/kg respectively. A theoretical calculation of HHV of fish oil is difficult due to the heterogeneous nature of the oil (polar to non-polar organics, variation by species etc…) [20]. However, if one assumes approximately 70-80 wt. % TAG and the balance phospholipids and fatty acids the HHV is approximately 38 MJ/kg, within the range of this study. The HHV of bunker fuel oil (43.36 MJ/kg) is in the range of biodiesel (39 MJ/kg to 43.33 MJ/kg) reported by Sivaramakrishnan et al. [11] and slightly lower than that of petroleum diesel (49.65 MJ/kg). The HHVs of the waste fish oil blends decreases as the ratio of waste fish oil increases (e.g. HHV-WB20 > HHV-WB50 > HHV-WB80). A similar trend is exhibited by the unrefined salmon oil blends with bunker fuel oil.

Table 3.2: Heating value of unrefined fish oil and blends

Samples	HHV (MJ/kg)	Flash point (°C)
Waste fish oil	38.69	203.5
SB20[a]	43.43	
SB50[a]	41.33	144
SB80[a]	40.46	
Unrefined Salmon oil	39.51	208.5
WB20[c]	42.19	
WB50[c]	41.20	145
WB80[c]	39.44	
RFO[b]	43.36	130
Cod oil	39.62	212.5
Mackerel	39.42	
Herring	39.71	

[a] SB20, SB50, SB80 = 20, 50 and 80 % (v/v) unrefined salmon oil blend with RFO
[b] RFO = Residual fuel oil
[c] WB20, WB50, WB80 = 20, 50 and 80 % (v/v) waste fish oil blend with RFO

3.3.3 Flash point testing

The flash point is an indicator of the flammability of compounds [21] and critical in developing safe handling, storage and use procedures. Biodiesel has a relatively high flash point, above 150 °C [22], while mineral diesel flashes between 55 °C and 66 °C [22]. CHRIS-hazardous chemical data [23] reported fish oil flash point at 215.56 °C, while Behcet [24] reported 216 °C and fish oil biodiesel to be 155°C. Detwiler and

Markley [25] determined that the flash, smoke and fire point of biological oils (e.g. soybean oil) varied considerably with the degree of refining, especially the removal of free fatty acids, and also with the mode of extraction. The flash point of the bunker fuel oil in this work is 130 °C, well below the flash point of the waste fish oil and the unrefined salmon oil of 203.5 °C and 208.5 °C respectively (Table 3.2). Detwiler and Markley [25] tested lightly processed Menhaden, light-deodorized fish oil, and kettle-refined-deodorized fish oil tested and obtained flash points of 302 °C, 298 °C, and 279 °C respectively. The flash points of the blends WB50 and SB50 are 145 °C and 144 °C respectively, lower than the flash points of unblended waste fish oil and unrefined salmon oil but slightly higher than the unblended RFO oil flash point. The blends flash points are of lower values than that of pure (B100) biodiesel but high enough from a safety perspective to be safely stored and transported at ambient temperatures in temperate regions. The lower flash points reported in this work may be due to the presence of free fatty acids in both fish oil (i.e 14.5 % and 6.6 % FFA of waste fish oil and unrefined salmon oil respectively). This is in agreement with Detwiler and Markley [25].

3.3.4. Melting points and enthalpy

The DSC thermograms (Figure 3.2a and 3.2b) show two broad endothermic peaks for the waste fish oil and the unrefined salmon oil respectively. The melting point of waste fish oil ranged from -21.07 to 20.25 ^{o}C, whereas the melting point of unrefined salmon oil ranged from -34.69 to 16.95 °C (Table 3.3a). The negative melting points of the oils were attributed to the presence of triacylglycerol which contain unsaturated fatty acids [8,10,26]. The higher degree of unsaturated fatty acids in fish oil result in melting at

lower temperatures, conversely, a higher degree of saturated fatty acids in fish oil result in high melting temperatures [8]. The ratios of polyunsaturated to saturated fatty acids in both waste fish oil and unrefined salmon oil are greater than unity (Table 3.3b).

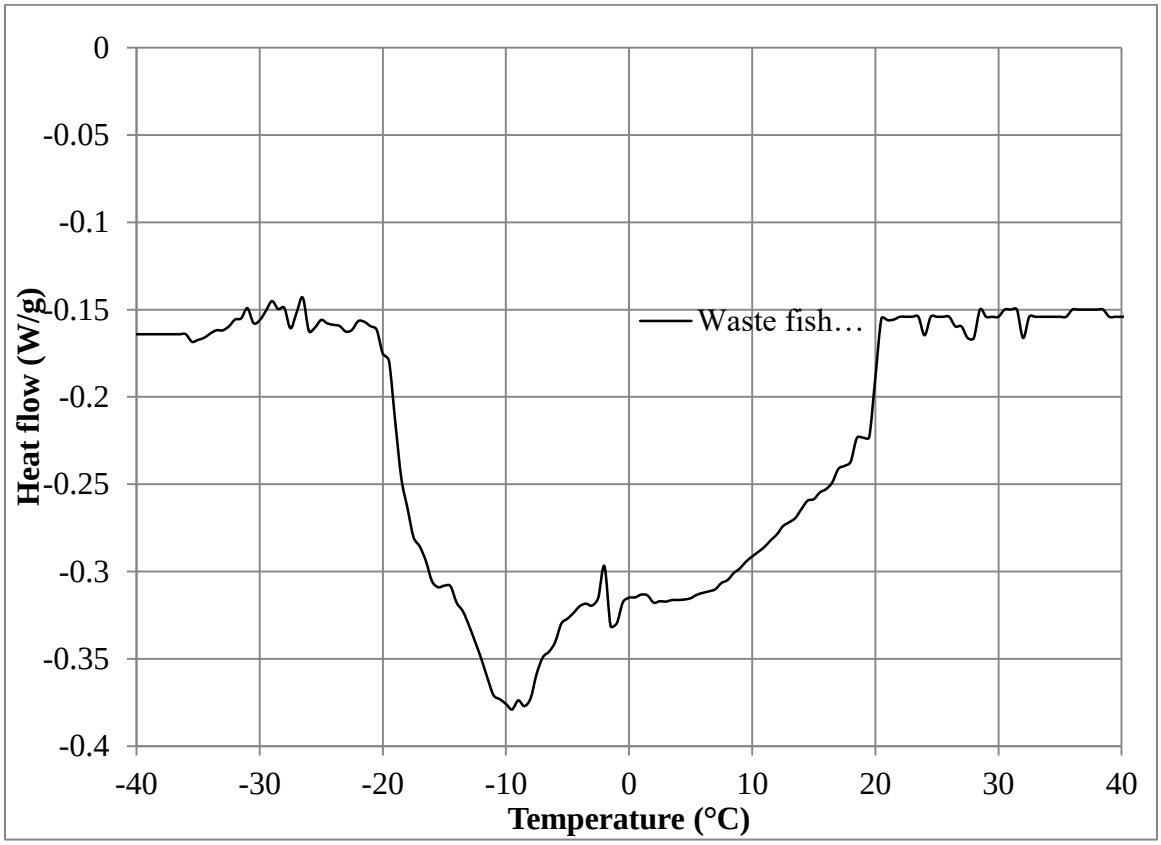

Figure 3.2a: DCS thermograph of the waste fish fish oil

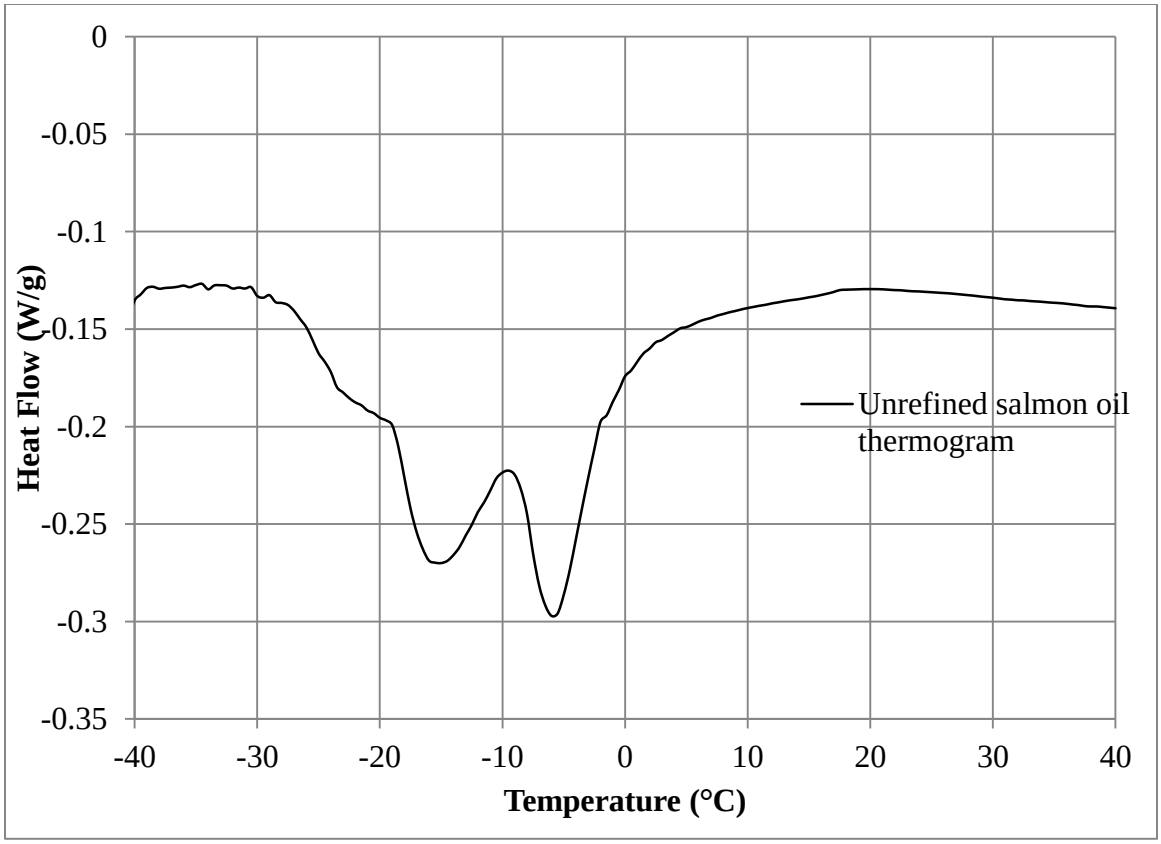

Figure 3.2b: DCS thermograph of unrefined salmon oil

The thermogram peaks for all the oils were not sharp due to the presence of impurities such as phospholipids, free fatty acids, aldehydes, particulate matters, ketones, water, and pigments. These impurities result in a melting behaviour typical of pure fatty acids [8,26]. Table 3.3a summarizes the enthalpy of the waste fish oil and unrefined salmon oil. The enthalpy of the waste fish oil is higher (51.80 kJ/kg) than that of unrefined salmon oil (33.43 kJ/kg). This indicated that for same amount of oil, more energy would be required for phase transition of the waste fish oil compared to unrefined salmon oil from solid to liquid state. The enthalpy of unrefined herring, mackerel and northern cod oil are 52.92, 53.60, and 41.45 kJ/kg respectively.

Table 3.3a: Melting points onset temperature and enthalpy of the oils

Sample	T_{on} (^oC)	T_{off} (^oC)	Peak(^oC)	Enthalpy(kJ/kg)
Waste Oil	-21.07 ± 2.21	20.25 ± 0.44	-9.98 ± 0.60	51.80 ± 2.53
Unrefined salmon Oil	-34.69 ± 0.46	16.06 ± 0.54	- 6.0 ± 0.08	33.43 ± 2.16
Herring	-18.37 ± 1.17	16.25 ± 0.40	-3.48 ± 2.63	52.92 ± 2.54
Mackerel	-29.31 ± 1.97	20.07 ± 0.15	-9.98 ± 0.45	53.6 ± 2.77
Northern Cod	-21.85 ± 1.51	12.16 ± 0.11	-15.58 ± 1.02	41.45 ± 1.12

T_{on} = Onset melting temperature; T_{off} = Offset melting temperature

Table 3.3b: Fatty acid composition of waste fish oil and unrefined salmon oil

	(wt %) from total	
Component	Waste fish oil	Unrefined salmon oil
Bacterial	1.82 ± 0.05	1.11 ± 0.04
Saturated fatty acids	21.76 ± 1.55	20.58 ± 0.08
MUFA[d]	21.64 ± 0.20	49.51 ± 0.02
PUFA[e]	55.57 ± 1.35	29.41 ± 0.07
PUFA/SFA[f]	2.56 ± 0.25	1.43 ± 0.01
ω-3[g] ratio	47.57 ± 1.07	11.58 ± 0.01
DHA/EPA[h] ratio	0.76 ± 0.02	0.71 ± 0.01

[d]MUFA = monounsaturated; [e]PUFA = polyunsaturated fatty acid;
[f]SFA = saturated fatty acid; [h]DHA = docosahexaenoic acid;
[h]EPA = eicosapentaenoic acid; [g]ω-3 = omega -3

The value of enthalpy reported for unrefined salmon in this work is lower compared to the 58.7 kJ/kg reported by [10], and 40 kJ/kg and 39 kJ/kg for red and pink salmon

respectively reported by [9]. The value disparities could be attributed to different in origin, harvesting season, and extraction method and quality of raw materials.

3.3.5. Rheological properties

The change in apparent viscosity with the shear rate with temperature was investigated. Results are summarized for unrefined salmon oil and the waste fish oil at the maximum temperature studied (60 ^{o}C), in Fig.3.3. At 60 ^{o}C the waste fish oil viscosity decreased from 0.059 Pa.s to approximately 0.0154 Pa.s when the shear rate was increased from 13 s^{-1} to 106 s^{-1} and remained constant after. At 60 ^{o}C the drop was from 0.0605 Pa.s to 0.0197 Pa.s for unrefined salmon oil when the shear rate was increased from 13 s^{-1} to 106 s^{-1} and then stabilized. Bunker fuel oil viscosity decreased from 0.1268 Pa.s to 0.0639 Pa.s and remained stable when the shear rate was increased from 13 s^{-1} to 92.4 s^{-1}. In the case of blends WB50 and WB50, the viscosity decreased from 0.082 Pa.s and 0.099 Pa.s to approximately 0.0303 Pa.s and 0.0352 Pa.s respectively, when the shear stress was increased from 13 s^{-1} to 66 s^{-1} and then stabilized. The effect of both waste fish oil and the unrefined salmon at 50 % (v/v) lowered the viscosity at 60 ^{o}C (Fig.3.3).

The decrease in viscosity with increasing shear rate indicated that the samples and their blends exhibited a shear-thinning non-Newtonian behavior which is in agreement with our previous work [20].

The flow behavior index (n) shows that the raw samples behave pseudoplastically (i.e $n<1$). The power law parameters are given in Table 3.4a and 3.4b. The flow behavior index (n) of waste fish oil and unrefined salmon oil at 20 ^{o}C are approximately 0.8 and 0.9 respectively, indicating non-Newtonian behavior [8,10]. These values are similar to

those reported for unpurified salmon oil by [8], and unrefined pollock oil by [10]. The n values were less than unity indicating that these products are pseudoplastic materials at all temperatures and blend concentrations studied. The coefficients (R^2) obtained were very close to unity, confirming that the power law model is suitable in describing flow. In practice, specific additives are added to viscous material to achieve pseudoplastic flow behavior. This behavior is beneficial to fuel flow through pipes and hoses as stress forces applied through pumps reduce fuel viscosity. The stronger the shear force applied to the pseudoplastic material, the greater the thinning effect and reduction in viscosity [27]. The pseudoplasticity of the raw samples and blends increases with temperature and concentration of fish oil in bunker fuel oil (Table 3.4a and 3.4b). The consistency coefficient, *k*, decreases with increases in temperature over the range of waste fish oil and unrefined salmon oil concentrations. This shows consistency with viscosity behavior and indicated that viscosity values decrease with increase in temperature at all fish oil concentrations in bunker fuel oil.

In eq. (2) the activation energy, E_a, (kJ/mol) represents the energy barrier to flow [8,10,13,28]. The frequency factor or viscosity coefficient at a reference temperature, *A*, (Pa.s) and E_a values were determined using a best fit line equation with least sum of square errors (SSE). The magnitude of the temperature effect on bunker fuel oil varied with fish oil concentration as shown by the *Ea* values in Table 3.5. In general, the higher the activation energy, the more sensitive the viscosity will be to temperature [28]. As shown in Table 3.5, the *Ea* decreased from 47.18 kJ/mol at 20 % (v/v) waste fish oil blend to 44.68 kJ/mol at 50 % (v/v) waste fish oil blend and to 35.75 kJ/mol of waste fish

oil at 80 % (v/v) waste fish oil blend. A similar trend was observed with unrefined salmon oil from 47.6kJ/mol at 20 % (v/v) to 35.37 kJ/mol at 50 % (v/v) and to 28.69 kJ/mol at 80 % (v/v). This is an indication that the temperature effect on viscosity is more dominant at lower fish oil blends.

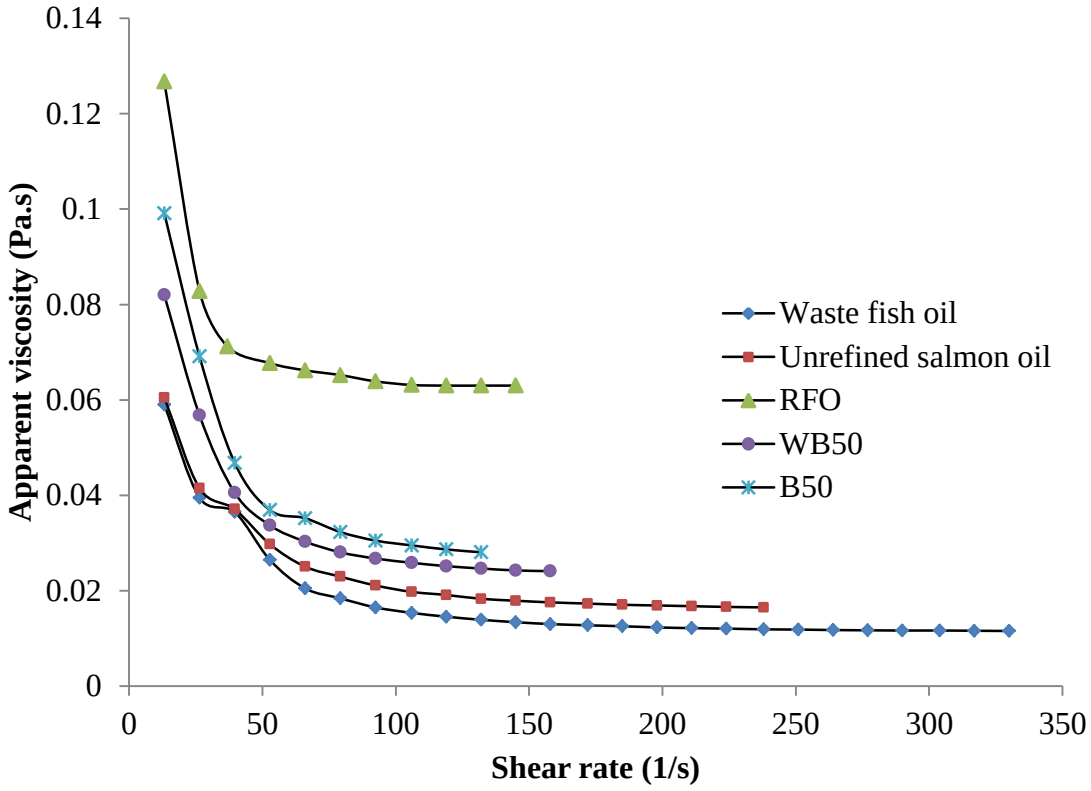

Figure 3.3: Change in apparent viscosity with shear rate at 60 ^{o}C for waste fish oil, unrefined salmon oil, RFO and their 50% (v/v) blends. RFO-residual fuel oil; WB50-50 % (v/v) waste fish oil blend with RFO; SB50-50 % (v/v) unrefined salmon oil blend with RFO.

Table 3.4a: Flow behavior index, consistency index, apparent viscosity, from power law equation fitting of waste fish oil, unrefined salmon oil, RFO and cod oil

Sample	T (^{o}C)	Viscosty (Pa.s)	n-values	K- values	R^2
Waste fish oil	20	0.1436 ± 0.001	0.81 ± 0.000	0.48 ± 0.000	0.995
	30	0.0646 ± 0.0002	0.71 ± 0.001	0.39 ± 0.007	0.998
	40	0.0166 ± 0.0001	0.79 ± 0.002	0.27 ± 0.001	0.996
	50	0.0125 ± 0.0000	0.53 ± 0.003	0.28 ± 0.008	0.996
	60	0.0089 ± 0.0000	0.48 ± 0.004	0.29 ± 0.011	0.988
Unrefined Salmon oil	20	0.0576 ± 0.0004	0.89 ± 0.007	0.04 ± 0.014	1.000
	30	0.0376 ± 0.0000	0.79 ± 0.006	---	0.999
	40	0.0246 ± 0.0002	0.69 ± 0.003	0.07 ± 0.004	0.998
	50	0.0169 ± 0.0001	0.50 ± 0.002	0.40 ± 0.000	0.992
	60	0.0127 ± 0.0002	0.53 ± 0.007	0.28 ± 0.012	0.996
RFO[b]	40	0.1448 ± 0.0073	0.72 ± 0.04	0.64 ± 0.060	0.998
	50	0.0711 ± 0.0003	0.54 ± 0.01	0.74 ± 0.020	1.000
	60	0.0483 ± 0.0014	0.55 ± 0.02	0.61 ± 0.040	0.989
Cod	20	0.05061 ± 0.0040	0.56 ± 0.007	0.57 ± 0.012	0.993
	30	0.03471 ± 0.0040	0.56 ± 0.044	0.47 ± 0.084	0.997
	40	0.02199 ± 0.0010	0.47 ± 0.000	0.56 ± 0.003	0.987
	50	0.01703 ± 0.0000	0.50 ± 0.003	0.44 ± 0.005	0.988
	60	0.01298 ± 0.0000	0.50 ± 0.034	0.36 ± 0.089	0.997

Table 3.4b: Flow behavior index, consistency index, apparent viscosity, from power law equation fitting of blends of waste fish oil and unrefined salmon oil with RFO.

		Waste fish oil blends			Unrefined salmon oil blends		
Parameter	T (^{o}C)	WB20[c]	WB50[c]	WB80[c]	SB20[a]	SB50[a]	SB80[a]
n-index	20			0.8±0.000			0.6±0.025
	30	0.8 ± 0.022	0.7 ± 0.000	0.6 ± 0.007		0.6 ± 0.012	0.4 ± 0.016
	40	0.7 ± 0.006	0.5 ± 0.032	0.5 ± 0.010	0.7 ± 0.001	0.5 ± 0.002	0.5±0.005
	50	0.6 ± 0.009	0.5 ± 0.001	0.5 ± 0.014	0.5 ± 0.001	0.4 ± 0.009	0.4 ± 0.017
	60	0.5 ± 0.002	0.5 ± 0.011	0.5 ± 0.013	0.4 ± 0.001	0.4 ± 0.006	0.5 ± 0.002
Viscosity (Pa.s)	20			0.13 ± 0.0002			0.07 ± 0.005
	30	0.16 ± 0.0080	0.07 ± 0.0009	0.05 ± 0.0011		0.07 ± 0.000	0.04 ± 0.001
	40	0.08 ± 0.0010	0.04 ± 0.0014	0.03 ± 0.0002	0.09 ± 0.001	0.04 ± 0.000	0.03 ± 0.000
	50	0.04 ± 0.0010	0.02 ± 0.0000	0.02 ± 0.0002	0.05 ± 0.000	0.03 ± 0.000	0.02 ± 0.001
	60	0.03 ± 0.0001	0.02 ± 0.0002	0.01 ± 0.0002	0.03 ± 0.000	0.02 ± 0.000	0.02 ± 0.000
K-values	20			0.43 ± 0.000			0.68 ± 0.054
	30	0.47 ± 0.016	0.45 ± 0.005	0.55 ± 0.002		0.64 ± 0.019	0.82 ± 0.030
	40	0.53 ± 0.008	0.52 ± 0.060	0.53 ± 0.023	0.58 ± 0.003	0.68 ± 0.003	0.64 ± 0.011
	50	0.55 ± 0.016	0.59 ± 0.001	0.43 ± 0.030	0.69 ± 0.003	0.65 ± 0.019	0.61 ± 0.031
	60	0.58 ± 0.004	0.45 ± 0.020	0.41 ± 0.029	0.73 ± 0.008	0.59 ± 0.008	0.49 ± 0.000

Table 3.5: The activation energy *Ea* of the raw oils and their blends

Sample	*E*a (kJ mol^{-1})	*A*(Pa.S)
Waste fish oil	58.884 ± 0.4497	$4.01x10^{-12}$
Unrefined salmon oil	31.036 ± 0.5661	$1.69\ x10^{-7}$
Cod oil	31.421 ± 1.3886	$1.49x10^{-7}$
SB20[a]	47.592 ± 2.0188	$1.14\ x10^{-9}$
SB50[a]	35.372 ± 0.0682	$5.28\ x10^{-8}$
SB80[a]	28.688 ± 2.6761	$6.14\ x10^{-7}$
RFO[b]	47.662 ± 2.0358	$1.7x\ 10^{-9}$
WB20[c]	47.178 ± 2.3633	$1.29\ x10^{-9}$
WB50[c]	44.667 ± 0.1581	$1.2\ x10^{-9}$
WB80[c]	35.749 ± 0.7977	$4.3\ x10^{-8}$

3.4 Summary and conclusion.

In this work the thermal and rheological properties of waste fish oil, unrefined salmon oil, bunker fuel oil and their blends have been analyzed. The feasibility of using unblended and/or blends of fish oil in conventional heaters/boilers/engines are determined by these properties. The fish oil, bunker fuel oil and their blends behaved as a shear thinning fluid and could be described by the power-law model. For bunker fuel oil /fish oil blends an increase in fish oil resulted in an increase in pseudo-plasticity of the blend. The activation energy decreased with increasing concentration of fish oil in blends.

Based on these results and previous studies in our lab [20], the blending of fish oil and bunker fuel oil fuel could potentially be used in home heating oil, steam/hot water - boilers, and marine fuels. The flash point of the blends is high enough for safe handling, transportation and storage. The fish oil/ bunker fuel oil blends have a lower heating value than the petroleum based fuel and therefore the ratio of fish oil: petroleum fuel must be considered. At high concentration of waste fish oils in the blends, the activation energy (flow barrier), E_a, becomes smaller and therefore exhibits good flow properties, which may be beneficial to applications in colder regions. The data on the rheological properties of the blends could be used as a tool in determining applications, storage and handling methods of the blends. Blending fish oils (biofuel) with the bunker fuel oil (petroleum based fuel oil) also minimize CO emission [3,29]; hence, this application may lessen the environmental concerns over emission properties of bunker fuel oil as fuel.

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CHAPTER 4

SOLUBILITY MEASUREMENT, EXPERIMENTAL AND MASS TRANSFER MODELING OF SALMON PROCESSING WASTE USING SC-CO_2

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Abstract

The method of extraction of oil for fuel from fish waste will determine the quality and applicability of the fuel. In this study, supercritical carbon dioxide extraction (SC-CO_2) was studied as a method to recover oil from salmon waste. Experiments at pressures of 15, 25, and 35 MPa, temperatures of 313, 333 and 353 K, and CO_2 flow rates of 0.18–0.48 kg/hr were conducted and the yield compared. The yields at 35 MPa, temperatures of 313, 333 and 353 K, and CO_2 flow rates of 0.18 kg/hr were approximately 39, 46 and 41 (wt.%). A process model based on intra-particle diffusion (D_e) and external mass transfer of fish oil (k_f) is presented for the supercritical extraction process. The adsorption equilibrium constant (K) is determined by fitting the theoretical extraction curve to the experimental data. The model using the best fit of theoretical extraction curve correlated the experimental data satisfactorily with AAD (%) ranged from 2.4 to 10.6 %.

The experimental and the mathematical model of SC-CO_2 extraction has been published in the *journal of Supercritical Fluids*. Mr. Ibraheem Adeoti is the lead author and Dr. Kelly Hawboldt is co-author. Mr. Adeoti's contributions to this paper include the following:

- Wrote the paper
- Performed all the laboratory testing and analyses (except where noted)
- Conducted all data processing and interpretation of results
- Performed all literature searches required for background information

Dr. Hawboldt provided technical guidance and editing of the manuscript.

Nomenclature

Abbreviations

AAD	Average absolute deviation
CASD	Center for aquaculture and seafood development
CO_2	Carbon dioxide
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EOS	equation of states
SCE	Supercritical carbondioxide extraction
SC-CO_2	Supercritical carbondioxide
SFE	Supercritical fluid extraction

Symbols

a_p	Specific surface area (m^{-1})
A	constant defined by eq. (25) (-)
b	constant defined by eq. (26) (-)
A_b	bed cross sectional area (m^2)
c	constant defined by eq. (27) (-)
c_f	solute concentration in the solvent phase (kg/m^3)
c_p	solute concentration within the particle pore (kg/m^3) c_p
c_{ps}	solute concentration in pore space at the particle surface (kg/m^3)
c_s	solute concentration in the particle (kg/m^3)

c_0 solute concentration in the pore phase at $t = 0$ (kg/m^3)

c_{s0} solute concentration in the solid phase at $t = 0$ (kg/m^3)

c_{f0} total solute concentration (kg/m3)

d_p particle diameter or thickness of the slab (m)

D_{ab} binary diffusion coefficient (m^2/s)

D_e effective intraparticle diffusion coefficient (m^2/s)

F cumulative fraction of solute extracted (-)

k_a adsorption rate constant (1/s)

k_f external mass transfer coefficient (m/s)

k_g global mass transfer coefficient (m/s)

K adsorption equilibrium constant (-)

L length of the bed (m)

Q_{CO_2} CO_2 volumetric flow rate (m^3/s)

r radial position in spherical particle (m)

R radius of spherical particle (m)

t time (s)

U_s superficial velocity (m/s)

x dimensionless solute concentration in effluent (-)

x_0 initial solute mass ratio in the solid phase (-)

x_p dimensionless solute concentration in pore (-)

x_s dimensionless solute concentration in solid phase (-)

y	solute mass ratio in the fluid phase (kg/kg)
z	bed length coordinate (m)
α	bed void fraction (-)
β	particle porosity (-)
ϕ	dimensionless mass transfer coefficient (-)
θ	dimensionless time (-)
ρ_s	solid density without void fraction of the solid matrix (kg/m^3)
ρ_{CO_2}	CO_2 density (kg/m^3)
τ	total bed volume (m^3)/volumetric flow rate (s)
ψ	particle geometry factor
ξ	particle geometry parameter

4.1 Introduction

Supercritical fluid extraction (SFE) is an alternative to conventional separation processes in the recovery of essential oils from plants and animal tissue. SFE using CO_2 (SC-CO_2) typically results in a high purity product and free of toxic solvent compared to conventional solvent extraction processes [1-3]. SC-CO_2 has a high organic diffusivity, is a relatively inexpensive solvent, and is nonflammable. In the SFE process the fluid is at its critical temperature and pressure, where it exhibits gas like viscosities and liquid like densities. Several investigators have explored SFE using carbon dioxide (CO_2) for the extraction of edible/nutraceutical oils from plant and animal products (e.g. peach almond seed [4,5], sunflower seed [6,7], canola [8], spearmint leaf [9], sesame seed [10], peppermint [11], aromatic plant [12], vetiver root [13], hazelnut [3], ginger [14], and fish [15-21]).

Fish processing facilities generate a significant amount of fish by-products that could be an important source of energy, food, or industrial feedstock. While fish oil is a natural source of omega-3 polyunsaturated fatty acids (mostly eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) used in nutritional supplements, the ability to extract, refine, and get to market may be challenging at processing facilities where there is limited infrastructure and plants are remotely located. Further, regulatory roadblocks of using oil from waste in food/drugs may be an issue. Under these conditions, extraction of oil from fish by-product for use as an in-house or as a regional fuel may be more sustainable. Processes to extract and refine fish oil for fuel are less energy intensive than the processes for nutritional quality oils and do not require the stringent product

specifications and approval process as in the food and pharmaceutical industry [22]. Unlike food crops, extraction of oil from fish residue does not negatively impact food production [22]. Fish oils from fish wastes are increasingly being used either as stand-alone or in blends with petroleum based fuels. The bio-oil composition and thermal and physical properties are a function of the feedstock and the degree of processing of the crude bio-oil.

Mathematical modelling of separation processes is critical in predicting process behavior, design of the process, and subsequent scale up [16,23]. In large scale SFE processes the solute concentration profiles in the solid and fluid phase are difficult to measure due to cost and the issues associated with sampling continuously in a high pressure system [23]. A process model based on experimental data could be used as a tool in scale-up and optimization of a SC-CO_2 process. Several models have been proposed for the SFE of fixed bed of solid substrate based on empirical kinetic equations [24-26]. The advantages of kinetic models are their simplicity and ability to describe the kinetics of an extraction profile very precisely. Kinetic studies have been used in the SFE of biomass [8,27-29]. del Valle et al., [24] presented a review of kinetic and equilibrium models of SFE processes. For scale up and design purposes, the kinetic models are not adequate as they fail to provide the description of underlying mass transfer phenomena and in these cases mass balance models can be used [24,26,30]. Many researchers have investigated mass balance models for SFE of a solute from fixed bed of solid substrate and del Valle et al., [24] provides a comprehensive review. Studies relevant to this work are summarized in Table 4.1. As Table 4.1 shows, there is little work on the extraction of oil and lipids from

animal muscle. In our laboratory, we are investigating SFE using CO_2 to extract fish oil from fish processing waste. There are several challenges with this type of work, predominantly in the heterogeneity of the waste material as fish species and degree of fish processing varies based on location and season. In Newfoundland and Labrador, salmon (*Salmo salar Linnaeus*) is harvested and processed. The high oil content and proximity to waste feedstock made this an ideal feedstock for our study. Offal (heads, trimmings and frames) discarded during peeling; cutting and evisceration processes were obtained from local industries. This study is part of an ongoing research on alternative fish oil extraction methods beneficial for biofuel application [22], low grade biofuel process optimization [51], comparison of biofuel quality as a function of oil extraction methods (currently under review - Fuel Journal), characterization of biofuel blends with petroleum fractions (currently under review - Fuel Journal), and life cycle analysis (LCA) of various extraction processes studied (currently being done).

This research study is divided into three folds: (1) fish oil solubility in SC-CO_2 was determined in the equilibrium experiment; (2) the SFE extraction process using CO_2 (optimize the SFE process conditions to maximize oil yield under the least intensive conditions; pressures, temperatures and CO_2 consumption); and (3) we developed a model predicting oil extraction as a function of process conditions.

Table 4.1: Examples of experimental and modeling work on supercritical extraction

Reference	Substrate	Solute	Model name
[12]	Aromatic plants	volatile oil	Grosso et al., and Coelho et al, model
[11]	Peppermint leave	Peppermint oil	Goto model
[31]	Black Pepper	Piperine	[a]IE mass transfer model
[32]	Black Pepper	Piperine	Sovova model
[33]	Black Pepper	Piperine	Sovova model
[9]	Spearmint leaf	Carvone and Limonene	[b]IBC-Diff model
[34]	Neem seeds	Nimbin	Goto Model
[35]	Nutmeg seed	Nutmeg oil	[c]BIC model
[36]	Nutmeg seed	Nutmeg oil	[d]SC/ADPF model
[37]	Carqueja plant	Essential oil	Sovova model
[8]	Canola seed	Canola oil	Unsteady state mass transfer model
[38]	Sage seed, Celery seed, Coriander seed	Essential oils	[e]IMTC model
[39]	Sage seed,	Essential oils	[g]LDF/PMMS model
[14]	Ginger root	Ginger oil	[f]SC model
[40]	Lavender flower	Essential oil	[f]SC/PF model
[41]	Clove buds	Essential oil	[gh]LDF/ADPF model
[42]	Clove buds and Vetiver root	Essential oil	Sovova model
[43]	Orange flower	Orange flower extract	[j]LDF/PF model
[7]	Sunflower seed	Essential oil	Kinetic model
[44]	Sunflower seed	Essential oil	Desorption model
[45]	pennyroyal leaves	Essential oil	[gh]LDF/ADPF

[46]	Marigold, fennel and chamomile	Essential oil	Micro scale model
[47]	Plane tree leaf	Mannitol	Axial dispersion/ Freundlich isotherm
[13]	Vetiver root	Essential oil	Axial dispersion
Animal/animal products			
[48]	Egg yolk	Lipids	Goto model
[16]	Mackerel	Fish oil	[a]IE/diffusion model
[19]	Trout powder	Fatty acids	Diffusion model
[49]	Cow brain	Cholesterol	Fick's diffusion model
[17]	Sardine	Lipids	N/A
[21]	Mackerel	Essential oil	N/A
[50]	Pigskin	Fat	N/A

[a]IE-internal +external mass transfer; [b]IBC-Diff-intact and broken cell diffusion; [c]BIC-broken and intact cell; [d]SC/ADPF-shrinking core/adsorption-desorption plug flow; [e]IMTC-internal mass transfer control; [f]SC/PF-shrinking core/plug flow; [g]LDF/PMMS-linear driving force/perfectly mixed multi-stages; [h]ADPF-Adsorption-desorption plug flow; [j]LDF/PF-linear driving force/plug flow.

4.2 Mathematical modeling

Freeze-dried salmon offal is made up of protein, bone meal, and moisture and a lipid-oil mixture. The lipid-oil mixtures are the extractable components and can be treated as a single (pseudo-solute) substance. The general mass transfer equations for the SFE processes proposed by Akgerman et al. [52], de Valle et al., [24] and de Valle et al. [53] are similar to mass transport operations involving solid-fluid processes, such as adsorption, desorption, and leaching. The models comprise of two differential solute mass balances on the solvent and solid phases. It also incorporates local equilibrium adsorption that describes the relationship between solute and solid [34]. The extraction model in this work is based on the model first developed by Goto et al. [11] for the prediction of essential oil extraction from peppermint leaves. Duford et al. [16] used the Goto et al. model to determine the cumulative fraction of edible oil from Atlantic mackerel at different moisture content and Mongkholkhajornsilp et al. [34] also used the model proposed by Goto et al. for the correlation of nimbin extraction from neem seeds. Rai et al. [25] compared Goto et al. model with models proposed by Reverchon, Marrone, Sovova and Goodarznia. The local adsorption equilibrium model proposed by Goto et al. [8] was found most suitable for our process as it addresses intra-particle diffusion and external mass transfer of SFE in a fixed solid bed.

The fixed bed of salmon offal particle containing the oil is defined as the stationary (solid) phase with flowing SC-CO2 as the mobile (fluid) phase, with assumptions that:

(i) the solid particle is porous,

(ii) axial and radial dispersions are negligible,

(iii) the process is isobaric and isothermal,

(iv) local equilibrium adsorption exists between solute and solid in pores of fish particle,

(v) solute interaction in the fluid or solid phase is negligible,

(vi) the SC-CO_2 physical properties (such as viscosity and density) are constant.

The solute concentration, c_f, in the fluid phase in a packed bed depends on the rate of mass transfer from particles along the length of the bed, the fluid flow rate, and extent of mixing. Eq. (1) is obtained by taking a mass balance around an element Δz of bed length as shown in Fig.4.1 [24]. The differential solute mass balance for the SC-CO_2 surrounding the fish particle in the packed bed is given as:

$$\alpha \frac{\partial c_f}{\partial_t} + U \frac{\partial c_f}{\partial z} = a_p (1-\alpha) k_f (c_{ps} - c_f) \qquad (1)$$

Where c_f and c_{ps} are solute (oil) concentration in the bulk SC-CO_2 phase and in the pore at the particle surface respectively and k_f is the external mass transfer coefficient of the particle, a_p is the specific surface area of the particle and is defined as the elemental, the bed packing characteristics, and solid substrate particle internal structure.

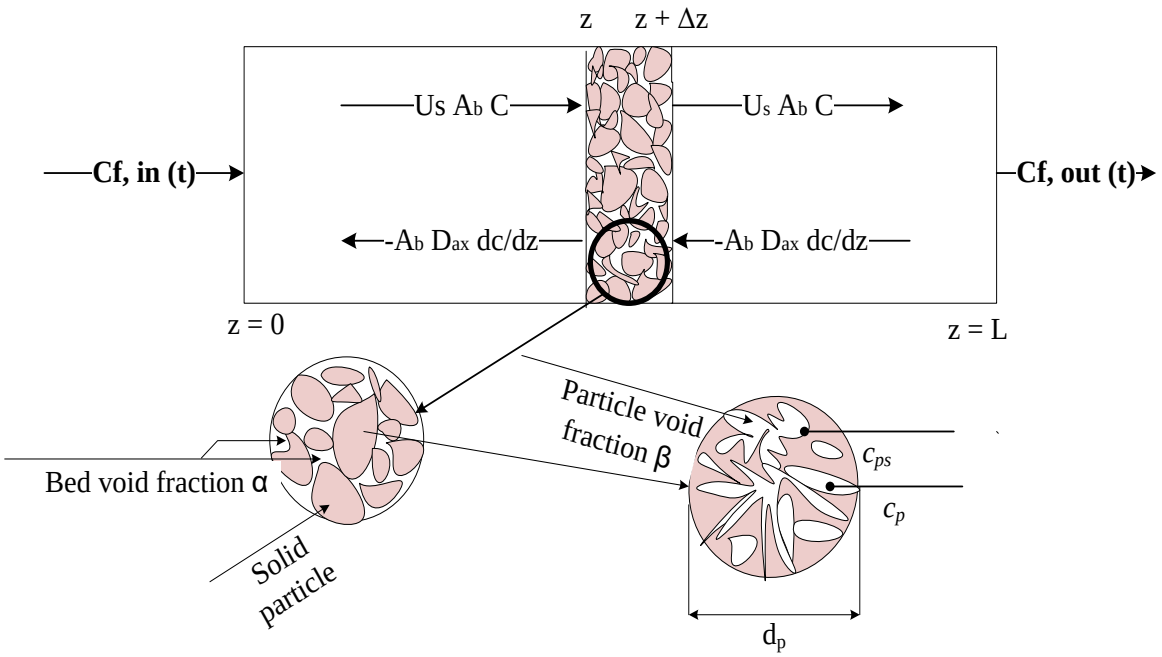

Figure 4.1: Flow diagram for fish waste packed bed extraction vessel showing finite difference volume element, the bed packing characteristics, and fish waste particle internal structure.

$a_p = \frac{\psi}{d_p}$. ψ is a factor that depends on particle geometry (ψ is 2 for very thin slab), and

d_p is thickness of the slab assumed in this research.

Initial conditions are:

$$c_f = 0 \, at \, z = 0 \tag{2a}$$

$$c_f = 0 \, at \, t = 0; 0 \le z \le L \tag{2b}$$

Boundary conditions are:

$$\frac{\partial c_f}{\partial z} = 0 \, at \, z = L \tag{3a}$$

$$D_{ax}\frac{\partial c_f}{\partial z} = Uc_f ; at\ z = 0\ for\ all\ t \tag{3b}$$

The differential solute mass balance in the solid phase is:

$$\beta\frac{\partial c_p}{\partial t} = De\frac{\partial c_p^2}{\partial r^2} - (1-\beta)\frac{\partial c_s}{\partial t} \tag{4}$$

Where c_p and c_s are the solute concentration in pores within particle and at the solid phase of particle respectively.

Initial conditions are:

$$c_p = c_0\ at\ t = 0, 0 \le r \le R \tag{5a}$$

$$c_s = c_{s0}\ at\ t = 0, 0 \le r \le R \tag{5b}$$

Boundary conditions are:

$$De\frac{\partial c_p}{\partial r} = k_f(c_f - c_{ps}), at\ r = R\ for\ all\ t \tag{6a}$$

$$\frac{\partial c_p}{\partial r} = 0, at\ r = 0\ for\ all\ t \tag{6b}$$

$$c_{f0} = \beta c_{p0} + (1-\beta)c_{s0} \tag{6c}$$

Where *De* is the effective intra-particle diffusion coefficient and d_p is the thickness of thin slab particle. The linear driving force (LDF) approximation for mass transfer from substrate to the SC-CO_2 is assumed to be valid when the residual solute concentration profile in the partially extracted solid substrate is approximately parabolic. The LDF approximation (Eq. 7a) was then used to combine internal and external mass process as [25,34,52]:

$$\frac{\partial c_f}{\partial t} = \frac{15 D_e}{R^2}(c_f - c_{ps}) \tag{7a}$$

$$k_f a_p (1-\alpha)(c_f - c_{ps}) = \frac{15 De}{R^2}(1-\alpha)(c_{ps} - c_f) \tag{7b}$$

Under these conditions the global mass transfer coefficient k_g, accounts for both the internal and external mass transfer resistances, and is given by:

$$k_g = \frac{k_f}{1 + \frac{Bi}{\xi}} \tag{8}$$

Where k_g is global (overall) mass transfer coefficient, ξ is the particle geometry parameter (ξ=10 for spheres and 6 for thin slabs), and *Bi* is dimensionless Biot number and is given as:

$$Bi = \frac{k_f d_p}{De} \tag{9}$$

The local extraction rate, which is equivalent to the desorption rate, is assumed to be reversible and linear in terms of adsorption rate constant k_a and the adsorption equilibrium constant *K*

$$\frac{\partial c_s}{\partial t} = k_a \left(c_p - \frac{c_s}{K} \right) \tag{10}$$

Average intra-particle and solid concentration are determined using the parabolic profile given as

$$\bar{c}_p = \frac{1}{2\pi R^2} \int_0^R 4\pi r^2 c_p(r) dr \tag{11}$$

$$\bar{c}_s = \frac{1}{2\pi R^2} \int_0^R 4\pi r^2 c_s(r) dr \tag{12}$$

In terms of average intra-particle concentration $\bar{c}_p$, the differential mass balance equations (1) and (4) can be reduced to

$$\alpha \frac{dc_f}{dt} + \frac{c_f}{\tau} = k_g a_p (1-\alpha)(\overline{c}_p - c_f) \tag{13}$$

$$\beta \frac{d\overline{c}_p}{dt} = k_g a_p (c_f - \overline{c}_p) - (1-\beta)\frac{d\overline{c}_s}{dt} \tag{14}$$

$$\frac{d\overline{c}_s}{\partial t} = k_a \left(\overline{c}_p - \frac{\overline{c}_s}{K} \right) \tag{15}$$

The average residual solute concentration in the solid in terms of instantaneous equilibrium in the pore space is given by:

$$\overline{c}_p = \frac{\overline{c}_s}{K} \tag{16}$$

The total initial equilibrium solute concentration exiting the solid phase at equilibrium is given by:

$$c_0 = \left[\frac{\beta}{K} + (1-\beta) \right] c_{s,0} \tag{17}$$

Equations (13) and (14) become:

$$\frac{dc_f}{dt} + \frac{c_f}{\alpha\tau} = k_g a_p \frac{(1-\alpha)}{\alpha} \left(\frac{\overline{c}_s}{K} - c_f \right) \tag{18}$$

$$\frac{d\overline{c}_s}{dt} = \frac{k_g a_p}{\left[\frac{\beta}{K} + (1-\beta) \right]} \left(c_f - \frac{\overline{c}_s}{K} \right) \tag{19}$$

The model equations can be further transformed into dimensionless forms: $x_0 = \frac{c_f}{c_0}$, $x_p = \frac{\overline{c_p}}{c_0}, x_s = \frac{\overline{c_s}}{c_0}$, $\theta = \frac{t}{\tau}, \phi = k_g a_p \tau$. Equations (18) and (19) in terms of dimensionless variables become:

$$\frac{dx}{d\theta} = \frac{\phi(1-\alpha)}{\alpha}\left(\frac{x_s}{K} - x\right) - \frac{x}{\alpha} \tag{20}$$

$$\frac{dx_s}{d\theta} = \frac{\phi}{\left[\frac{\beta}{K} + (1-\beta)\right]}\left(x - \frac{x_s}{K}\right) \tag{21}$$

Initial conditions are:

$$x = 0 \ at \ \theta = 0 \tag{22a}$$

$$x_s = \frac{1}{\left[\frac{\beta}{K} + (1-\beta)\right]} \ at \ \theta = 0 \tag{22b}$$

Equation (20) and (21) are the dimensionless ODE for the solute concentration in the bulk fluid and solid phase respectively. They are used with initial conditions (22a) and (22b). These equations are simplified using Laplace transform to obtain an analytical solution of the model in terms of the dimensionless solute concentration in the bulk fluid phase given by equation (23), as:

$$x(\theta) = A[\exp(\gamma_1\theta) - \exp(\gamma_2\theta)] \tag{23}$$

where,

$$\gamma_1 = \frac{-b + \sqrt{b^2 - 4c}}{2}, \ \gamma_2 = \frac{-b - \sqrt{b^2 - 4c}}{2} \tag{24}$$

$$A = \frac{\phi(1-\alpha)}{[\beta + (1-\beta)\mathrm{K}]\alpha(\gamma_1 - \gamma_2)} \tag{25}$$

$$b = \frac{\phi}{\beta + (1-\beta)K} + \frac{\phi(1-\alpha)}{\alpha} + \frac{1}{\alpha} \tag{26}$$

$$c = \frac{\phi}{[\beta + (1-\beta)K]\alpha} \tag{27}$$

The cumulative fraction of solute extracted up to dimensionless time θ is given by:

$$F(\theta) = \frac{1}{(1-\alpha)} \int_0^{\theta} \mathrm{x}\, d\theta = \frac{A}{(1-\alpha)} \left[\frac{\exp(\gamma_1 \theta) - 1}{\gamma_1} - \frac{\exp(\gamma_2 \theta) - 1}{\gamma_2} \right] \tag{28}$$

Equation (28) gives the extraction yield and combined with experimental data to determine the constants defined by equations (24), (25), (26), and (27). When the mass transfer resistance is negligible, that is, $\theta = \infty$, equation (28) reduces to:

$$F(\theta) = 1 - \exp\left\{ \frac{\theta}{\left[\beta + (1-\beta)K(1-\alpha) + \alpha\right]} \right\} \tag{29}$$

The mass ratio of the solute in the fluid phase as a function of time can be determined by:

$$y(t) = \left[\frac{\beta}{K} + (1-\beta) \right] \frac{\mathrm{x}_i \rho_s}{\rho_{CO_2}} A \left[\exp\left(\gamma_1 \frac{t}{\tau} \right) - \exp\left(\gamma_2 \frac{t}{\tau} \right) \right] \tag{30}$$

where $\mathrm{x}_i = \dfrac{\rho_{CO_2} y_i}{c_0}$

The mass of extract at the bed outlet at any time *t* can be determined by:

$$m(t) = \int_0^t y(\mathrm{t})\, \mathrm{Q}_{CO_2}\, \rho_{CO_2} dt \tag{31}$$

Combining equations (24) and (25) into equation (31) becomes

$$m(t) = \left[\frac{\beta}{K} + (1-\beta)\right] \mathrm{x}_0\, \rho_s\, \mathrm{Q}_{CO_2}\, A\tau \left\{\frac{1}{\gamma_1}\left[\exp\left(\gamma_1 \frac{t}{\tau}\right) - 1\right] + \frac{1}{\gamma_2}\left[1 - \exp\left(\gamma_2 \frac{t}{\tau}\right)\right]\right\} \qquad (32)$$

4.3 Materials and methods

The feedstock was a by-product of the fish industry; specifically the offcuts (offal) from farmed salmon (*Salmo salar Linnaeus*), (harvested during winter season 2013) from Cooke aquaculture, and provided by the Centre for Aquaculture and Seafood Development (CASD). The offal consisted of salmon heads, trimmings, and frames, discarded during peeling, cutting and evisceration processes in the fish plant. The by-product collected 'as is' was frozen at -40 °C and kept frozen until the experiments were performed. The sample was crushed (using a Hobart grinder manufacturing Co. Ltd. Don Mills, Ontario Canada) to (5–10 mm equivalent diameter) substrate suitable for freeze drying. The crushed sample was freeze dried at 0.133 bar vacuum and -47 °C for 24 hours. The dried samples were blended using a Ninja professional blender (NJ-600 series 1000W Euro-Pro Ninja® USA) to produce fine particles range of 125 to 710 µm with average particle diameter of 418 µm. Supercritical grade carbon dioxide (4.8 - 99.99%) was provided by Praxair Co., Canada. The obtained grounded sample contained approximately 7- 10.03 % (wt. %) moisture content, 42.6 - 52.8 % (wt. %) fish oil, and 21.6 - 23.8% protein. The total fraction of oil was determined by solvent (soxhlet) extraction using hexane.

Soxhlet extraction was used as a comparison to determine the total oil content of the sample as a baseline. Five grams of freeze dried samples were treated with 150 cm^3 of

hexane for 8 hours using (Pyrex glass soxhlet apparatus). The extracted oil was evaporated under vacuum at 65 °C using a rotary evaporator and then dried in the oven at 40 °C for 1.5 hours and cooled in desiccators before reweighing.

The experimental setup for the solubility and the supercritical fluid extraction (SFE) process is shown in Fig. 4.2. The SFE apparatus consisted of Teledyne Isco Syringe pump D-series (model 260D, Teledyne Isco Inc., USA) fitted with a cooling jacket to cool the CO_2 and equipped with a reservoir to store or deliver liquid CO_2. The CO_2 was cooled with a cooling jacket attached to the syringe pump.

4.3.1 Solubility determination

The solubility of fish oil in SC-CO_2 was measured by loading crude fish oil adhered to silica beads (with an average diameter of 3 mm) into the extraction vessel (Penn Manufacturing Inc., 10,000 Psig @194°F, USA), and the vessel connected to SFE fluid delivery system (Fig.4.2). The extraction vessel and delivery tubes were wrapped with heating tape (Omega Engineering, Inc., USA; model HTWC101-010) to keep the system at the specified temperature. The solubility was performed at temperatures ranging from 313 to 353 K and pressures from 15 to 35 MPa. The liquid CO_2 was compressed to the desired pressure and then pumped to the extractor. Once the specified temperature and pressure were achieved, the heated flow-restriction valve was closed and this marked the start of solubility. All experiments were performed in triplicate and conducted with an equilibrium time of 2 hours, as determined in the preliminary tests. After 2 hours the flow-restriction was opened and oil was recovered in a collector. The cumulative mass of

CO_2 used in extraction was determined by the gas flow meter and totalizer. The solubility was determined in each case from the slope of weight of oil collected (g) versus total weight (g) of CO_2 that passed through the pressure column. The same approach was used to determine the solubility of waste-based oil except that the freeze dried waste was loaded into the vessel instead of crude oil and silica beads.

4.3.2 Supercritical fluid extraction (SFE)

In each experimental run, 5-8 g of sample (dry basis) was loaded into a 13.6 cm^3 extraction vessel (I.D. - 1.974 x 10^{-2} m, height – 4.46 x 10^{-2} m, Penn Manufacturing Inc., 10,000 Psig @194°F, USA), and the vessel connected to SFE fluid delivery system. The extraction vessel and delivery tubes were wrapped with heating tape (Omega Engineering, Inc., USA; model HTWC101-010) to keep the system at the specified temperature. The extractions were performed at temperatures ranging from 40 to 80 °C and pressures from 15 to 35 MPa. The liquid CO_2 was compressed to the desired pressure and then continuously pumped to the extractor at the specified flow (0.18-0.48 kg/hr). Once the specified temperature and pressure were achieved, the heated flow-restriction valve was opened and this marked the start of extraction. The exiting fluid stream with fish oil flowed at a constant flow rate, and was depressurized in a collector. The tubing and separator were washed with hexane at the end of each run to collect all extracted oil not recovered during the experiment. The cumulative mass of CO_2 used in extraction was determined by the gas flow meter and totalizer – XFM series (Aalborg Instruments & Controls, Inc. USA). The collected extracts were weighed at the end of each experiment, and stored in a freezer at -30 °C. Each experiment was carried out for 120 minutes and

sampled at 15 minute time intervals. The solubility of fish oil in SC-CO_2 was determined from a plot of extraction yield versus total CO_2 usage.

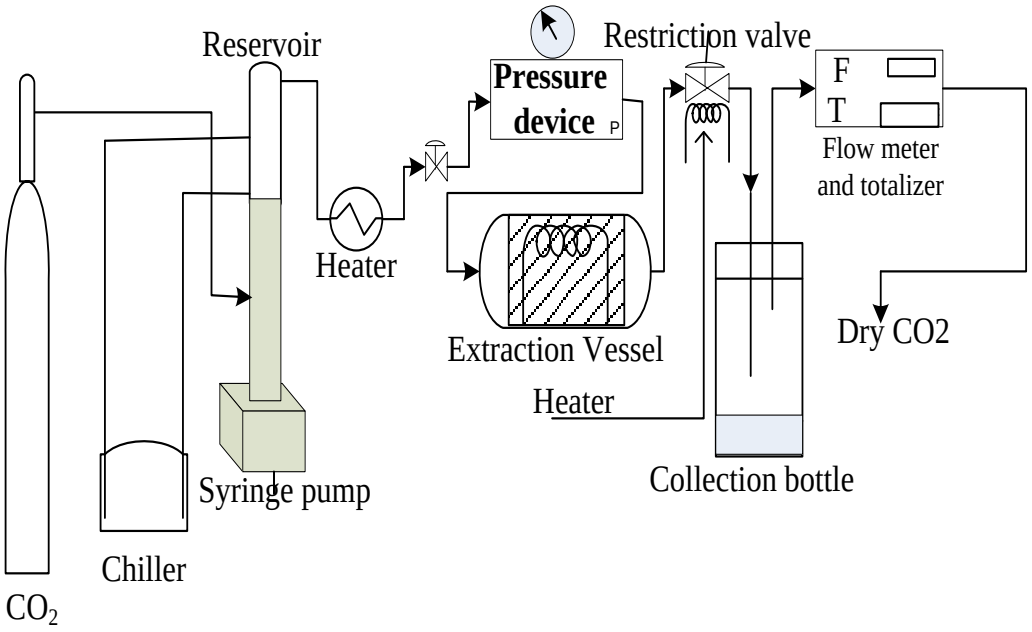

Figure 4.2: Schematic diagram of experimental apparatus (supercritical CO_2 experimental setup).

The experiments were conducted in triplicates. Mean and standard deviation were calculated using Microsoft Excel 2007 (Window 8 Edition, Microsoft Corporation, USA). Analysis of variance (ANOVA) at alpha level of 0.05 was performed using the statistical tool in Microsoft Excel. Average of three measurements plus/minus standard deviation was given in tables where applicable.

4.3.3 Model parameters estimation

The total oil concentration in the waste (c_s) was determined using Soxhlet extraction. The physical properties of SC-CO_2 such as density ρ and viscosity μ under the operating conditions are determined using the NIST database [54]. The binary diffusion coefficient of fish oil-CO_2 was estimated as a function of reduced temperature and density, molecular weight (M=885.4g/mol), and molar volume at the critical point using the Catchpole and King [55] as;

$$D_{12} = 5.152 D_c T_r (\rho_r^{\frac{-2}{3}} - 0.4510) \frac{R}{X}, \quad 1 < \rho_r < 2.5 \tag{33}$$

Where

$$D_c = 4.30x10^{-7} \frac{M_1^{0.5} T_c^{0.75}}{\sum_v^{2/3} \rho_c} \quad = 4.937 \text{ x } 10^{-8} \text{ (for } CO_2\text{)} \tag{34}$$

$$X = \frac{\left[1 + \left(\frac{V_{c2}}{V_{c1}}\right)^{1/3}\right]^2}{\left(1 + \frac{M_1}{M_2}\right)^{0.5}} \tag{35}$$

The correction factor *R* is defined as the ratio of binary to self-diffusion coefficients as follows:

$$\begin{aligned} R &= 1.0 \pm 0.1, \qquad X > 2 \\ R &= 0.664 X^{0.17} \pm 0.1, \qquad 2 < X < 10 \end{aligned} \tag{36a}$$

Therefore simplified equation for the diffusion coefficient correlation can be given as;

$$D_{12} = 2.544x10^{-7} D_c T_r (\rho_r^{\frac{-2}{3}} - 0.4510) \frac{R}{X} \tag{36b}$$

D_c is the self-diffusion coefficient of CO_2 at the critical point (4.9368 x 10^{-8} m^2/s), T_r is the reduced temperature, T_c is the CO_2 critical temperature, $\sum_v$ is diffusion volumes, ρ_r is the reduced density, V_c is the molar volume at the critical point, M is the molar mass, subscript 1 refers to CO_2, and subscript 2 refers to triolein. The mass transfer coefficient, k_f, is obtained using the equation due to Wakao and Kaguei [56]:

$$Sh = 2 + 1.1\mathrm{Re}^{0.6} Sc^{0.3} \tag{37}$$

where

$$Sh = \frac{d_p k_f}{D_{12}} \tag{38}$$

$$Sc = \frac{\mu_1}{D_{12}\rho_1} \tag{39}$$

$$\mathrm{Re} = \frac{d_p U_s \rho_1}{\mu_1} \tag{40}$$

The effective intra-particle diffusion coefficient was estimated using $D_e = D_{12}\beta^2$ [11,25,34]. The superficial velocity, U_s, was estimated from the ratio of solvent volumetric flow rate to the bed cross sectional area. The fish cake porosity β, was determined using a mercury porosimeter (Micromeritics Instrument Corporation, Auto-pore IV). The bed void fraction α was estimated from the volume of fish cake and bulk volume of bed.

4.4 Results and discussion

4.4.1 Solubility of fish oil

The solubility data of salmon oil in carbon dioxide measured at 313 – 353 K and 15 – 35 MPa were presented in Table 4.2a in comparison with the solubility of other fish and vegetable oils. The results show that the solubility of the fish oil increased with increasing pressure at constant temperature (Fig. 4.3) and the effect of temperature on fish oil solubility is less significant than the effect of pressure. For example, increase in pressure from 25 to 35 MPa increased the solubility by 10 %, 67% and 210 % at 313, 333 and 353 K respectively. At lower pressure (P ≤ 20MPa) the effect of solvent density dominated the fish oil solubility (Table 4.2a). When the temperature was increased from 313 to 353 K at 15 MPa, the fish oil solubility decreased from 17 to 8 g oil/kg CO_2. At pressure above 20 MPa, the effect of pressure dominated the effect of solvent density. The highest value for salmon solubility found in this study was 48.0 g oil/kg CO_2 at 35 MPa and 353 K. Catchpole et al. [57] reported the same value for squalene oil at 30 MPa and 333 K, and Suares et al. [58] reported 51 g oil/kg CO_2 for seed oil at 35 MPa and 333K (Table 4.2a).

The solubility values recorded in this study are in agreement with trend reported for squalene by Catchpole et al. [57], and for seed oil reported by Suares et al. [58]. The solubility data were correlated using Chrastil [59] equation as follows;

$$\ln S = k \ln \rho + \frac{a}{T} + b \qquad (41)$$

Where S is the solute solubility (g oil/kg CO_2), T is temperature (K), ρ is the density of pure SC-CO_2 (kg/m^3), and a, b and k are adjustable constants that can be evaluated from

experimental data. The linear regression of the experimental data yields the equation (42) as follows;

$$\ln S = 3.142 \ln \rho - \frac{3520.2}{T} - 7.18 \quad (42)$$

Equation (42) is consistent with those correlated by Catchpole et al. [57], Soares et al. [58], and Gucülu -Ustundagˇ and Temelli [60] (Table 4.2b).

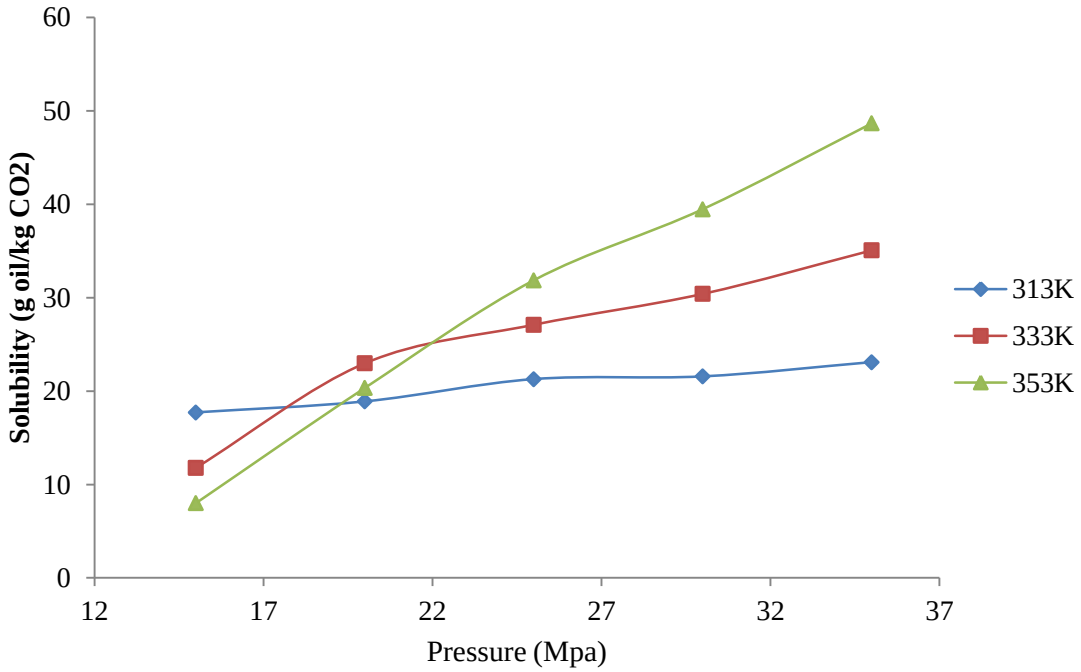

Figure 4.3: Salmon oil solubility as a function of temperature (K) and pressure (MPa)

Table 4.2a: Salmon oil solubility as compared to the solubility of other fish and vegetable oils

Solubility (g oil/kg CO_2)		This work		Catchpole et al.[57]				Soares et al. [58]	
Temperature (K)	Pressure (MPa)	Salmon oil in waste	Crude salmon oil	Squalene	cod liver oil	Spiny dogfish	Orange roughy oil	Babassu oil	Ucuuba oil
313	15.0	2.69 ± 0.07	17.72 ± 1.76						
	20.0		18.90 ± 2.1		3.66			20.9 ± 2.10	11.0 ± 0.93
	22.5				4.79				
	25.0	5.93 ± 0.05	21.29 ± 2.71		5.94			30.9 ± 0.42	18.1 ± 0.58
	30.0		21.58 ± 2.79					43.5 ± 2.16	24.6 ± 0.67
	35.0	10.63 ± 0.04	23.10 ± 3.25					51.4 ± 3.47	32. 7 ± 2.01
325	10.0			0.27					
	15.0			10.63					
	17.5			17.38					
	20.0			23.84					
	22.5			30.12					
333	15.0	1.62 ± 0.01	11.78 ± 1.17						
	20.0		22.98 ± 1.00		1.6	1.4	5.7	9.8 ± 0.09	7.4 ± 0.00
	22.5			27.05	2.54	2.02			
	25.0	6.11 ± 0.22	27.10 ± 2.12	34.65	3.92	2.95		21.2 ± 1.16	14.3 ± 0.82
	26.0						12.21		
	27.5			43	5.27	4.09			
	30.0		30.41 ± 3.11	47.7	7.08	5.4	17.1	39.7 ± 0.69	24.6 ± 1.05
	35.0	12.49 ± 0.13	35.07 ± 4.62					50.9 ± 1.89	38.0 ± 1.32
353	15.0	1.05 ± 0.62	8.00 ± 2.26						
	20.0		20.34 ± 1.70					4.8 ± 1.05	3.17 ± 0.70
	25.0	4.46 ± 0.62	31.87 ± 1.95					12.5 ± 0.47	8.65 ± 0.29
	30.0		39.48 ± 3.80					29.1 ± 0.48	18.9 ± 1.47
	35.0	10.84 ± 0.31	48.67 ± 3.22					46.3 ± 1.44	36 ± 4.89

Table 4.2b: Correlation constants of this work compare to literature values

Solute	T (K)	K± Standard error	a± Standard error	b± Standard error	R2	Reference
Samon oil	313-353	3.142 ± 0.2	-3520.2	-7.1839	0.998	This work
Squalene	314 - 333	7.3 ± 0.2	-4131.0	-33.0	0.9996	[60]
(Babbasu)	313-353	8.4049	-3419.8	-49.546		[58]
Squalene	313 - 333	6.54	-3936.6	-28.28		[57]

4.4.2 Dynamic behavior of extraction of fish oil

Fig.4.4 outlines the typical extractive curve (total extracted fish oil (g) versus total SC-CO_2 passed through the column (g)). The initial straight line indicates a constant rate of fish oil extraction. During the constant rate period, the external surfaces of the solid particles are likely covered with oil and the SC-CO_2 is close to saturation; therefore, fish oil extraction at this initial period is solubility controlled. This is followed by a transition period, during which the rate of extraction falls rapidly likely due to depletion of the layer of oil on the fish particle surface. At this point, the oil extraction rate is mass transfer controlled. The oil diffusion rate from the interior to the surface of the particles is low compared to the initial mass transfer rate. Lee et al. [8] reported similar trend for seeds oil at all extraction conditions tested where the initial constant extraction rate is characterized by solvent saturation, and is followed by a transition period, during which the extraction rate falls rapidly.

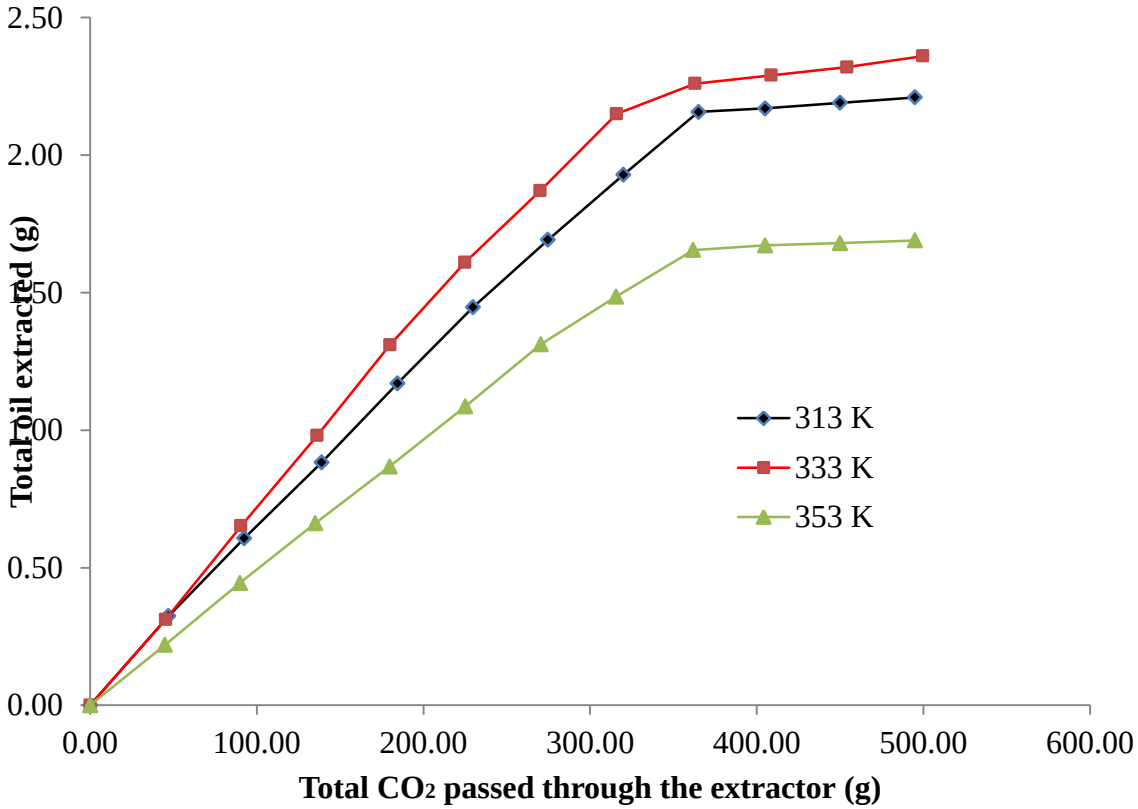

Figure 4.4: Typical extractive curve of experimental data at 0.18 kg/hr., 25 MPa and 353 K.

4.4.3. Goto et al. model compared with experimental data from this work.

Experimental data was compared with predictions from the tuned Goto et al. model. The errors were quantified by average absolute deviation, (AAD) % as defined below.

$$\text{AAD (\%)} = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{y_{experimental}-y_{estimated}}{y_{experimental}}\right|_i \text{ x } 100 \qquad (43)$$

Where N is the number of data points, $y_{experimental}$ and $y_{estimated}$ are the data obtained from experiments and model equations respectively. The AAD between the estimated cumulative extraction yields and experimental data are presented in Table 4.3. The AAD

for the fit ranged between 2.40 to 10.60 % indicating good match. The model was tested using a regression analysis of the data. The regression coefficient was 0.99. The probability was tested at 95 % confidence level and P-value <0.005 in both cumulative fraction and % yield, which indicate an insignificant deviation between the Goto et al. model and experimental results.

4.4.4. Effect of operating parameters on extraction yield

The effects of temperature, pressure and solvent flow rate on the calculated cumulative extraction yield as compared with experimental data are investigated in this section.

4.4.4.1. Effect of temperature on cumulative extraction yield.

Fig. 4.5 summarizes extraction at 313 - 353 K, 35 MPa and 0.18 kg/hr. At constant pressure, the cumulative extraction yield (F) increases with increasing temperature up to 333 k and drops thereafter. The plot of cumulative extraction yield (F) against time (t) represents the yield of fish oil at these conditions. As temperature increases, the solubility of oil in SC-CO_2 increases (Table 4.2 and Fig 4.3) due to the increase in oil vapour pressure. Güçlü-Üstündag and Temelli [61] reported at constant pressure, any increase in the temperature decreases the solvent density and increases the solute vapor pressure. From the analysis of Table 4.3, the estimated mass transfer coefficient (k_f) increases slightly with temperature (from 3.68 x 10^{-5} m/s at 313K to 5.56 x 10^{-5} m/s at 353K) as a result of increase in molecular diffusivity, as also reported by Rai et al. [25]. Similarly, the effective diffusivity (D_e) and the adsorption equilibrium (K) increase with increasing temperature. Thus the rate of mass transfer of solute to bulk solvent phase increases with increase in temperature.

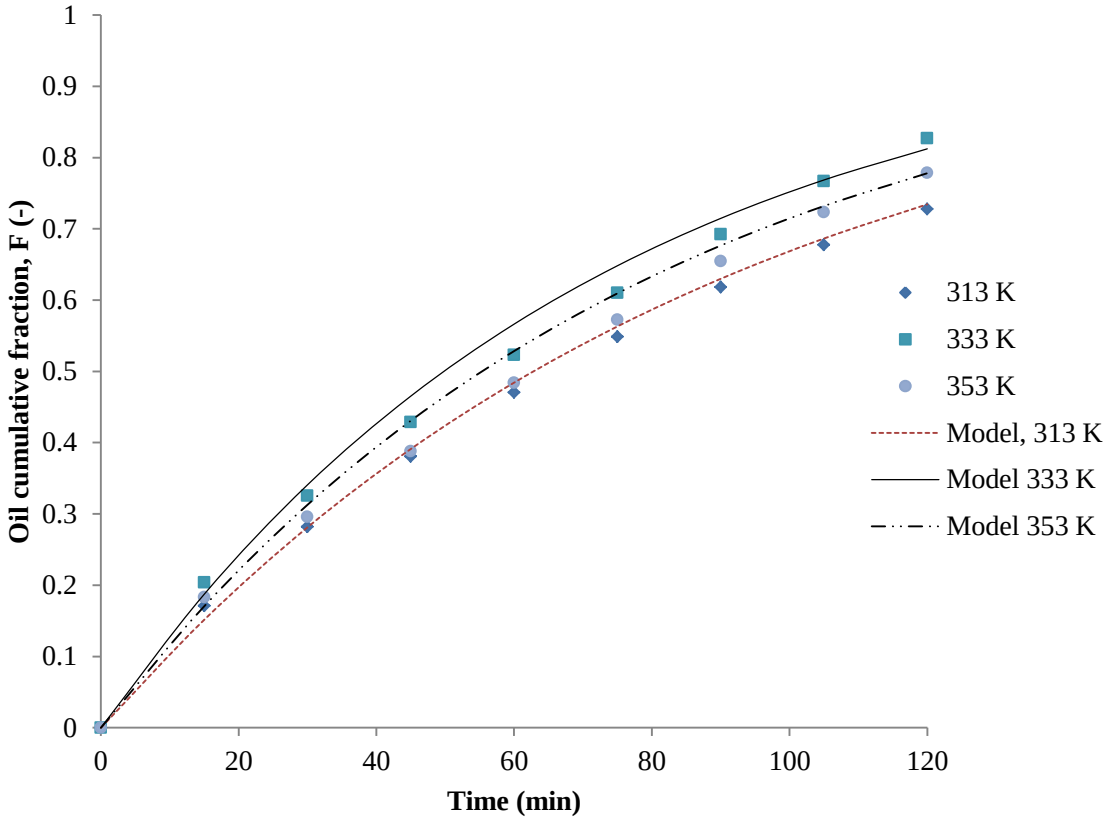

Figure 4.5: Effect of temperature (313 – 353 K) on extraction yield at 0.18 kg/hr and 35 MPa.

4.4.4.2. Effect of pressure on cumulative extraction yield.

The effect of an isothermal (333 K) increase in pressure on the cumulative extraction yield at 0.18 kg/hr solvent mass flow rate is presented in Fig.4.6. The increase in pressure from 15 to 35 MPa resulted in an increased solvent density (from 610 to 860 kgm^{-3}; Table 4.3), which in turn increases the oil solubility rapidly (from 1.62 to 12.49 g oil/kg CO_2; Table 4.2) and corresponding increase in yield (from 12.7 to 81%; Fig.4.6). High pressure has a positive effect on extraction process. Nei et al., [19]. Dunford et al., [16] proposed the oil extraction rate from Atlantic mackerel was controlled by solubility limitations

Table 4.3: Experimental conditions and the model process parameters at various for fish oil extraction

T (K)	P (MPa)	ρ (kg/m^3)	x10^{-9} *De* (m^2/s)	x10^{-5} μ (Pa.S)	x 10^{-7} *Q* (m^3/s)	*Re* (-)	x10^{-5} k_f (m/s)	Bi (-)	x10^{-6} k_g (m/s)	τ (s)	*K* (-)	ϕ(-)	AAD (%)	d_p (mm)
Effect of temperature														
313	35	905	0.677	10.0	0.552	0.68	3.68	22.65	6.66	246.16	192.4	7.83	3.01	0.4175
333	35	860	0.803	8.4	0.581	0.81	4.39	23.82	7.89	233.92	257	8.84	5.13	0.4175
353	35	785	1.02	7.0	0.637	0.97	5.56	22.74	9.99	213.52	306.2	10.22	5.52	0.4175
Effect of pressure														
333	15	610	1.44	4.7	0.82	1.45	8.24	23.84	14.29	165.92	3448	11.35	10.60	0.4175
333	25	785	0.96	6.9	0.637	0.99	5.31	23.11	9.44	213.52	895	9.66	6.60	0.4175
333	35	860	0.803	8.4	0.581	0.81	4.39	23.82	7.89	233.92	257	8.84	5.10	0.4175
Effect of flow rate														
313	35	905	0.677	10.0	0.552	0.68	3.68	22.65	6.66	246.16	192.4	7.83	3.20	0.4175
313	35	905	0.677	10.0	1.105	1.36	4.80	29.51	6.96	123.08	192.4	4.09	2.40	0.4175
313	35	905	0.677	10.0	1.470	1.81	5.42	33.32	7.07	92.31	192.4	3.12	3.10	0.4175
Effect of particle size														
353	35	785	1.02	7.0	0.637	0.23	15.28	15.03	38.14	213.52	306.2	162.88		0.100
353	35	785	1.02	7.0	0.637	0.97	5.56	22.74	9.99	213.52	306.2	10.22	4.41	0.4175
353	35	785	1.02	7.0	0.637	1.66	3.98	27.80	6.07	213.52	306.2	3.65		0.710
353	35	785	1.02	7.0	0.637	2.33	3.26	32.07	4.40	213.52	306.2	1.88		1.000

a_p = 4790.42 m^{-1}, average particle diameter d_p = 0.418 mm, β = 0.463, α = 0.62, and c_0 = 0.487 g/g of fish cake.

rather than intra-particle diffusion resistance. However, as the pressure increases from 15 to 35 MPa (Table 4.3), the mass transfer coefficient (k_f), the effective diffusivity (D_e) and the adsorption equilibrium (K) decreased. Rai et al. [25] reported a decrease in the mass transfer coefficients and the effective diffusivity with increased pressure. The authors proposed this was due to an increase in the mass transfer resistance with increasing pressure which shows a negative effect of pressure on extraction. Hence, the increased cumulative yield observed can be attributed to solubility dominance on the extraction rate as the pressure increases at constant temperature.

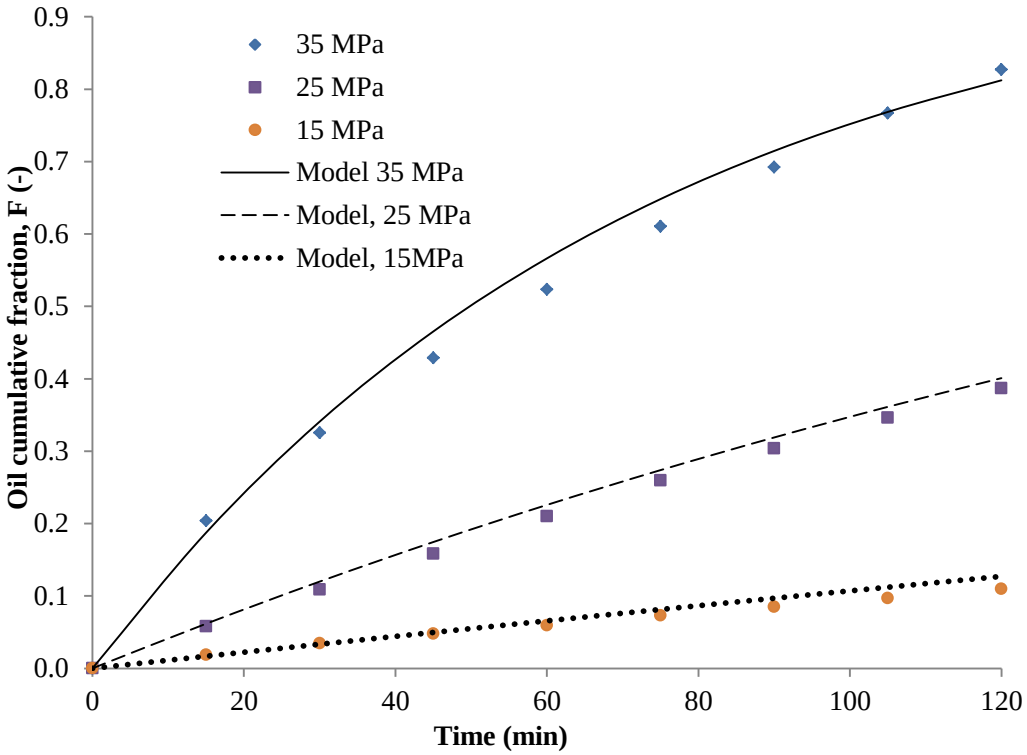

Figure 4.6: Effect of pressure (15 – 35 MPa) on extraction yield at 0.18 kg/hr and 333 k.

4.4.4.3. Effect of solvent mass flow rate on cumulative extraction yield.

Fig.4.7 shows the effect of solvent mass flow rate on extraction yield at 35 MPa and 313 K. The cumulative extraction yield, F, increases with an increase in solvent mass flow rate. As demonstrated in the extraction curves, the extraction rate increases with solvent surface velocity within the fixed bed (Fig.4.7). The extraction curves moves to the left along with solvent flow rate, which means that the overall extraction is faster. This is a result of a higher mass transfer coefficient which dominates over the reduced residence time. This observation can also be explained by the fact that the mass transfer coefficient increases with increasing solvent flow rate and thus the solute transferred per unit time to bulk liquid phase increases, leading to high extraction yield. The analysis of the estimated model parameters presented in Table 4.3 also shows that despite the decreased resident time (τ) from 246.16 s to 96.31 s as the CO_2 mass flow rate increased from 0.18 to 0.48 kg/hr, the mass transfer slightly increases from 3.68×10^{-5} to 5.42×10^{-5} m/s. This is also true for the dimensionless Reynolds number (*Re*) which also increased from 0.68 to 1.81. The increased mass transfer is likely due to better mixing due to increased turbulence.

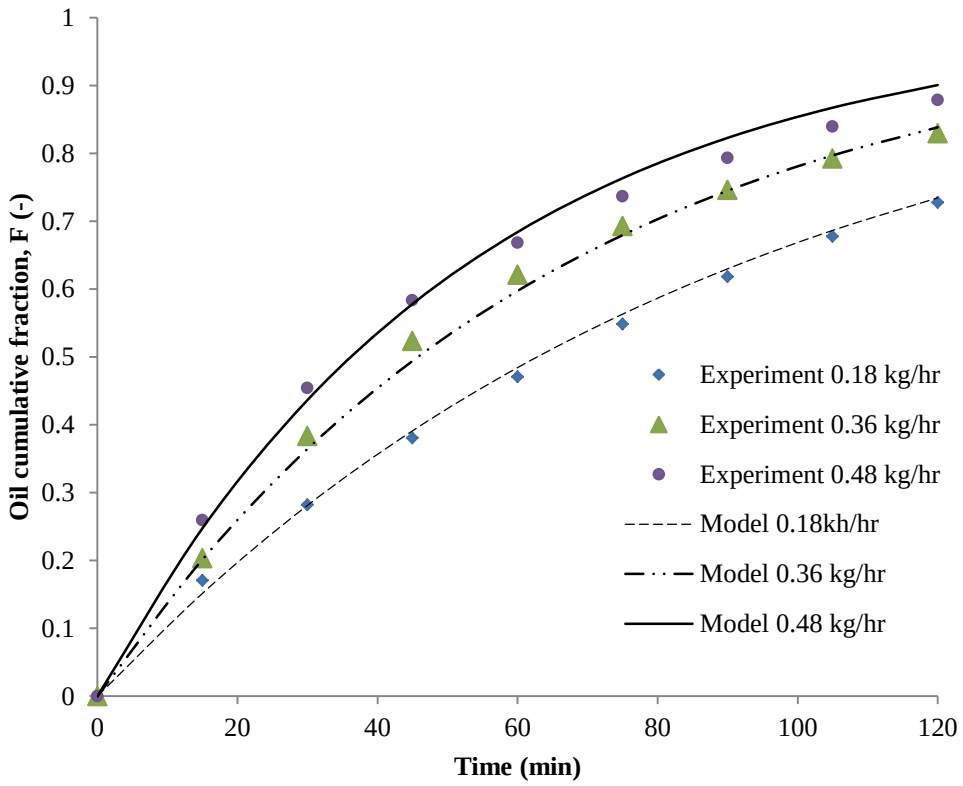

Figure 4.7: Effect of solvent mass flow rate on extraction yield at 313 K and 35MPa.

4.4.4.4. Predicted effect of particle size on cumulative extraction yield.

The effect of particle size (1.0, 0.71, 0.418, and 0.125 mm) on cumulative extraction yield is shown in Fig.4.8. As particle size increases the cumulative extraction yield decreases. The grinding process increases surface area and may disrupt the fat cell membrane, reducing mass transfer resistance, leaving the oil more accessible to the solvent. Furthermore, the intra-particle diffusion path and diffusion time become shorter for smaller particle sizes, causing an increase in the rate of diffusion, thus increase oil extraction yield. This effect may be stronger with smaller particle sizes as observed in

Fig.4.8. Similar observation trends were also noted in literature with vegetable and seed oils [10,25,34].

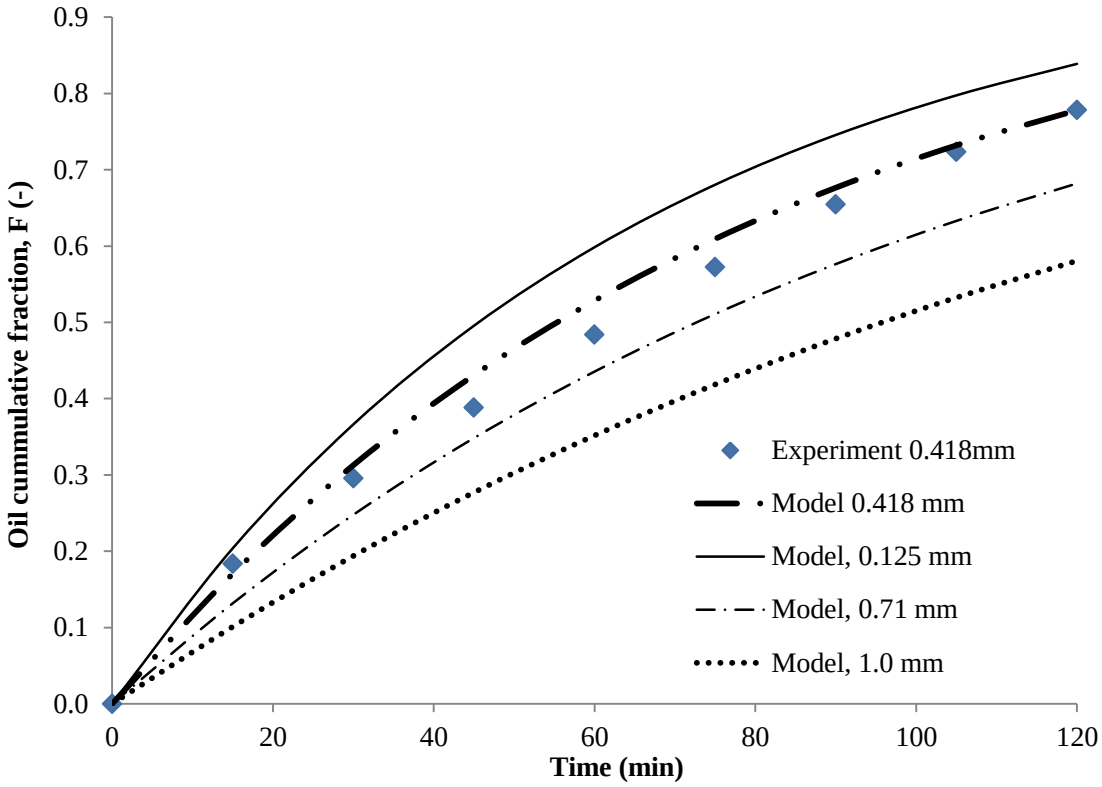

Figure 4.8: Predicted effect of particle size on extraction yield at P = 35 MPa, T = 353 K and mass flow rate 0.18 kg/hr.

4.5 Conclusion

Extraction of fish oil from salmon (*Salmo salar Linnaeus*) waste/discard (heads, trimmings and frames) by SC-CO_2 was studied. There have been limited studies on the SFE of fish oil from waste materials. The yield increases with increasing temperature (313 - 333 K), pressure (15 - 35 MPa), and CO_2 mass flow rate (0.18 - 0.48 kg/hr) for 2

hours experimental runs. The effect of pressure has a more significant increase in extraction yield compared to temperature and solvent flow rate. Decreasing particle size increases extraction yield. Comparison between experimental data and Goto et al. model produced a good match with AAD (%) ranged from 2.4 to 10.6 % indicating the model could be used for scale-up and design. Extraction of oil from fish waste could provide the fish plants or region with a source of locally produced bio-fuel. If the SFE process was paired with a process (such as a power plant or fish harvester) producing waste CO_2 this could enhance the economics of the process. This is the first stage of a study; the overall goal of the work is to determine the sustainability of SFE of fish oil from waste using a life cycle assessment approach. In particular we will evaluate the balance between high pressures and temperatures (which favor oil extraction) and the costs associated with these processes. For instance, extracting an extra 1% of oil may not be justified if there are significant costs associated with a high pressure or temperature.

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CHAPTER 5

COMPARISON OF BIOFUEL QUALITY OF WASTE DERIVED OILS AS A FUNCTION OF OIL EXTRACTION METHODS

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Abstract

Fish derived bio-oils have similar properties to petroleum-derived fuel oils and therefore the potential to be an alternative energy source. The quality of bio-oil as a fuel is determined by the quality of the feedstock and processing conditions. Fish oil may have poor cold flow properties due to the heterogeneity of the lipid composition. Different oil extraction methods produce different levels of homogeneity with respect to lipids. In this study, oil was extracted from fish waste via three different processes; modified fishmeal (MFM), supercritical extraction using carbon dioxide (SC-CO_2), and soxhlet extraction. The quality of oils extracted (composition, thermal degradation, physicochemical, and flow properties) were compared. The SC-CO_2 extracted 91% and the MFM extracted 71 % of the total oil contained in the fish waste. The SC-CO_2 oil is more than 86 wt % triglycerides, representing more homogeneous oil than the MFM at 70wt% and soxhlet at 66 wt %. The free fatty acid (FFA) of SC-CO_2 oil is lower than MFM and soxhlet oil, making it a better feedstock for biodiesel production. Polar lipids were most abundant in the soxhlet oil at 22.98 wt %, followed by the MFM oil at 18.35 wt % and SC-CO_2 oil at 7.39 wt %. The MFM oil exhibited a shear-thinning non-Newtonian behavior, while the SC-CO_2 oil was Newtonian. Overall, the oil from SC-CO_2 showed better fuel properties, particularly as a blend and/or replacement for heating oil, than the MFM and soxhlet oil and the process has the potential for a lower environmental footprint.

This chapter has been published in the *Fuel Journal* Mr. Ibraheem Adeoti is the lead author and Dr. Kelly Hawboldt is co-author. Mr. Adeoti's contributions to this paper include the following:

- Wrote the paper
- Performed all the laboratory testing and analyses (except where noted)
- Conducted all data processing and interpretation of results
- Performed all literature searches required for background information

Dr. Hawboldt provided technical guidance and editing of the manuscript.

Nomenclature

ALC	Alcohol(s)
ASTM	American Society for Testing and Materials
AMPL	Acetone mobile polar lipid(s)
ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
CASD	Centre for Aquaculture and Seafood Development
DAG	Diacylglycerol(s)
DHA	Docosahexaenoic acid(s)
DSC	Differential scanning calorimeter
EE	Ethyl ester(s)
EPA	Eicosapentaenoic acid(s)
GC-FID	Gas chromatography with flame ionization detection
GE	Glycerol ether(s)
EK	Ethyl ketone(s)
FAME	Fatty acid methyl ester(s)
FFA	Free fatty acid(s)
HC	Hydrocarbon(s)
HHV	High heating value
HPLC	High performance liquid chromatography
ID	Internal diameter
ME	Methyl esters(s)

MFM	Modified fishmeal
MK	Methyl ketone(s)
MUFA	Monounsaturated fatty acid(s)
MUN	Memorial University of Newfoundland
PL	Phospholipid(s)
PUFA	Polyunsaturated fatty acid(s)
SC-CO_2	Supercritical carbon dioxide
SFA	Saturated fatty acid(s)
SFE	Supercritical fluid extraction
SE	Steryl ester(s)
ST	Sterol(s)
TAG	Triacylglycerol
TGA	Thermo-gravimetric analysis
TLC-FID	Thin-layer chromatography with flame ionization detection

5.1 Introduction.

Fish processing operations generate considerable quantities of edible and inedible by-products. Approximately 45 wt % of the total catch of fish is discarded as processing byproduct including heads, frames, trimmings, fins, skin and viscera (gut, liver, etc…) [1]. Fish oil recovered from fish residue varies considerably (between a mass fraction of 1.4 % and 40.1 %) depending on the species, tissue [2] and season. There is an increasing interest in obtaining edible fish oil from fish by-products in order to satisfy the demand of omega-3 enriched products. However, waste fish oils can have a low-value application (fuel oil/ biodiesel) when the content of omega 3 fatty acids (EPA and/or DHA) or when the yield after the refining process are low [3]. Various studies have investigated waste fish oil as fuel for conventional combustors or diesel engines [4-7]. Fish oils have similar properties to petroleum-derived fuel oils such as calorific/heating value and combustion efficiency [5,8-10]. When compared to petroleum based fuels, biofuels have the advantage of lower toxicity, higher biodegradation rates (reducing impact in soil and water if spilled), no sulfur, and a higher flash point [5,8,11,12]. The quality of bio-oil as a fuel is determined by the quality of the feedstock and the processing conditions, which need to be carefully managed to obtain a high quality fuel [8,13]. Atabani et al. [14] showed biodiesel quality is a function of feedstock fatty acid composition, production, and refining method(s). Studies have demonstrated crude fish oil has poor cold flow properties such as lower lubricity and viscosity, and higher acidity compared to conventional diesel fuel [12]. This is because unrefined fish oil contains impurities such as free fatty acids (FFA), primary oxidation products, minerals, pigments,

moisture, and phospholipids [15]. High FFA and/or phospholipid levels reduce fish oil fuel quality and additional refining processes are required such as neutralization and degumming. Phospholipids polymerize due to heat and form deposits that clog injectors, valves, build up on the combustion chamber walls and cylinder surfaces in engines [13,16]. Sediments may clog fuel filters and pumps [13,17]. As a result of poor storage conditions of feedstock, the hydrolysis of triglycerides in the presence of water leads to high FFA and results in low oil stability during storage [18] and corrosion during use [13,18]. Water in fuel oil decreases the heating value, impedes ignition and slows down flame propagation [13].

The presence and/or quantity of impurities are highly dependent on the fish oil extraction method [15]. Fish oil (edible and non-edible) can be recovered through several methods. The wet reduction process is one of most common process employed in high volume fish oil production but may require subsequent refining steps in order to improve the fish oil quality [19]. Other conventional fish oil recovery processes use either high temperatures and/or flammable or toxic solvents, which could result in loss of functional properties and deterioration of oil quality [8,20-22]. Supercritical fluid extraction (SFE) has been proposed in the extraction of high quality compounds from natural sources [23] including oil recovery from seeds/biomass, whole fish and/or fish by-products [24]. SC-CO_2 for oil recovery is an attractive option as it is a non-toxic, non-flammable, inexpensive and clean solvent [8,22-25].

The objective of this study is to investigate the quality of oil extracted from fish waste using SC-CO2 process compared to conventional processes. Salmon oil extracted from

fish processing waste via three different methods; SC-CO2, soxhlet, and modified fishmeal (MFM) was compared. Chemical composition, rheological or flow properties and thermal stability were compared to determine their feasibility as a fuel oil.

5.2 Materials and methods

The materials were previously discussed in section 2.0 of chapter 3 and section 3.0 of chapter 4.

5.2.1 Oil recovery methods

The detailed methodology for modified fishmeal extraction method (MFM) was described in section 2.0 of chapter 3, soxhlet extraction method in section 3.0 of chapter 4 and the supercritical carbon dioxide extraction method (SC-CO2) in section 3.2 of chapter 4.

5.2.2 Lipid and fatty acid composition analysis

The lipid composition of oil was determined by thin-layer chromatography with flame ionization detection analysis (TLC/FID) as described in Parrish [27] and Deibel et al. [28]. Approximately 1 g of oil was extracted with 8 mL of chloroform, 4 mL of methanol, 4 mL of 2:1 chloroform: methanol, and 2 mL of chloroform extracted water in the first wash, and 6 mL of chloroform in 2-3 additional washes. The extracted lipids were diluted in chloroform and the solutions were analyzed for composition of fourteen lipid classes in the TLC/FID (Mark VI Iatroscan with silica coated chromarods). The lipid classes were straight chain hydrocarbons (HC), steryl esters (SE), ethyl esters (EE), methyl esters (ME), ethyl ketones (EK), methyl ketones (MK), glycerol ethers (GE), triacylglycerols (TAG), free fatty acids (FFA), alcohols (ALC), sterols (ST),

diacylglycerols (DAG), acetone mobile polar lipids (AMPL), and phospholipids (PL). The results were expressed in percentage weight of each lipid class in the extracted solution by including the dilution factor.

***Fatty acid composition analysis*:**

To derivatise the lipid, 250 μL extracted lipid from each samples injected into cleaned glass vials and 0.5 mL of hexane and 1mL of 14% BF3/ methanol were added. Glass vials were sealed with teflon tapes, vortexed for 30 s, sonicated for 4 min and oven heated for 1.5 hours at 85 °C. After heating, 0.5 mL of chloroform extracted water and 1.5 mL of hexane were added. The upper organic layer (FAME) was transferred to glass vials and concentrated to 1 mL. Fatty acids were analyzed on a GC/FID (HP 6890) equipped with an autosampler (7683) and a column ZB wax+ (length = 30 m, ID = 0.32 μm, Phenomenex, U.S.A). The final column temperature set at 220 °C and H_2 carrier gas flowing at 2 mL/min. Peaks were identified using retention times from standards purchased from Supelco, 37 component FAME mix (product number 47885-U), bacterial fatty acid ester mix (product number 47080-U), polyunsaturated fatty acid (PUFA) 1 (product number 47033) and PUFA 3 (product number 47085-U). The quantitative standard (product number GLC490) was purchased from Nu-Chek Prep, Inc. Chromatogram peaks were integrated using the Varian Galaxie Chromatography Data System (version 1.9.3.2).

5.2.3. Thermo Gravimetric Analysis (TGA)

The thermal stability of the salmon oils was determined using Thermo-gravimetric Analysis – TGA (Model Q500, TA Instruments Inc.). Approximately 0.8 - 1.2 mg of oil

sample was loaded into the furnace. The TGA was manually programmed to heat up the sample from ambient to 800℃ under N_2/air atmosphere at a ramping rate of 5 ℃/min. Sample weight change was automatically acquired every second and the data were analyzed and plotted using the TA Universal Analyzer Software.

5.2.4. Calorific value/High heating value (HHV)

Calorific value is a measure of the energy content in a fuel. The 1108 oxygen bomb calorimeter (Parr Instrument Company) was used to determine the HHV of the salmon oils according to ASTM D2015 standard method. Oxygen – bomb vessel was pressurized to approximately 3 MPa with an oxygen container. The bomb was ignited automatically after the jacket and a bucket temperature equilibrates to the desired values.

5.2.5. Melting points and enthalpy

Melting points were determined in a METTLER TOLEDO DSC-1 (Differential Scanning Calorimeter) with Julabo intercooler and TA Universal Analyzer Software. Approximately 5-10 mg of sample was cooled to -60 ^{o} and heating to 40 ^{o}C at 5 ^{o}C/min. The thermogram plots containing normalized heat flow with temperature were integrated to obtain peak onset and areas. The average melting points of the salmon oils was analyzed and recorded

5.2.6. Dynamic viscosity using the Brookfield Rheometer

The apparent viscosity of the salmon oils were measured using a Brookfield DV-III Ultra Programmable rheometer equipped with a small sample adapter and spindle (SC4-18/13RP). The temperature of the sample was kept constant by connecting a circulating water bath (NESLAB EX series) to a water jacket of the small sample adapter.

Measurements were taken at 20, 40, and 60 ℃ in (Pa.s) by changing the spindle rotation speed from 10 rpm at intervals of 10 until the torque exceeded 100%. Kinematic viscosity values of the oil samples are estimated using ν = μ / density, where, ν is kinematic viscosity of product or oil (cSt), μ is dynamic viscosity of product or oil samples (cP). The power law (Eq.1) was used to analyze the flow behavior of all the raw samples and their blends.

$$\tau = K\gamma^n \quad (1)$$

Where τ = shear stress (Pa.s), γ = shear rate (s^{-1}), K = consistency index ($Pa.s^n$) and n is the flow behavior index (dimensionless unit) [29-31]. Taking the logarithm of both sides of Eq.(1) yielded a straight line plot of log τ versus log γ with intercept (log K) and slope n. The use of Arrhenius relationship to describe the effect of temperature on apparent viscosity was through the use of Eq. (2) [30-32].

$$k = A.\exp.^{(-Ea/RT)} \quad (2)$$

Where k is the reaction rate constant, A is the frequency factor, Ea is the activation energy (J/mol), R is the gas constant (8.314 J/mol.K), and T is the temperature (K). A plot of natural logarithm (ln) of apparent viscosity versus inverse of temperature ($1/T$) was constructed for each of unrefined salmon oil, waste fish oil, and their blends. The slope of the straight line, the intercept and the regression coefficient were determined using the trend line of the plot. E_a was also calculated from the slope.

5.2.7. Water and sediments, moisture content, FFA/acid value, peroxide value, and density.

AOCS official standard method Ca 3d-02 was used to determine water content on the fish waste and measures the free water relative to the waste overall mass (gross mass including water). AOCS official standard method Ca 2e-84 for moisture content was used to determine residual moisture in the freeze dried waste. AOCS official standard method Ca 5a-40 was used to determine % FFA, AOCS official standard method Cd 8b-53 for peroxide value, and ASTM standard test method D1217-93 using pycnometer for density.

Statistical Analysis

The experiments were conducted in triplicates. Mean and standard deviation were calculated using Microsoft Excel 2007 (Window 8 Edition, Microsoft Corporation, USA). Analysis of variance (ANOVA) at alpha level of 0.05 was performed using the statistical tool in Microsoft Excel. Average of three measurements plus/minus standard deviation was given in tables where applicable.

5.3 Result and discussion.

5.3.1. Percentage recovery

The soxhlet method is standard for oil extraction and was used as a baseline to determine the effectiveness of both the MFM and SC-CO_2 methods. Previous work [22,33] indicates moisture content for SC-CO_2 extraction should be less than 10 wt %. If the water content is too high it can decrease the extraction efficiency and therefore the ground fish waste was freeze dried to 8.50 wt % (dry basis). The freeze dried waste was used for the SC-

CO_2 experiments and as a comparison a freeze dried sample was processed using soxhlet. In the MFM process, the oil is extracted from water laden waste through a series of heating and centrifuging. In this comparison, the fresh ground fish waste moisture content was 59.32 wt % (wet basis). A second set of soxhlet experiments were performed on this "wet" waste to determine the maximum oil extraction possible under "wet" conditions.

The Soxhlet method extracted 53 g of oil ± 0.73/100 g of dry fish waste while the SC-CO_2 process extracted 48.14 g of oil ± 1.06/100 g of dry fish waste or 91 wt% of the maximum oil. For the fresh ground fish waste, the Soxhlet extracted 28.37 g of oil ± 1.20/100 g of fish waste (wet basis), while the MFM extracted 20.70 g of oil ± 2.76/100 g of fish waste (wet basis), or 71 wt%. On a percentage basis, the SC-CO_2 is able to recover more of the oil compared to the MFM. The maximum recovery was achieved at CO_2 mass flow rate of 0.36 kg/hr, 333 K, and 35 MPa (Fig. 5.1). The SC-CO_2 oil recovery is in agreement with Rubio-Rodríguez et al. [25] who reported 96 wt % recovery of oil from hake offcuts.

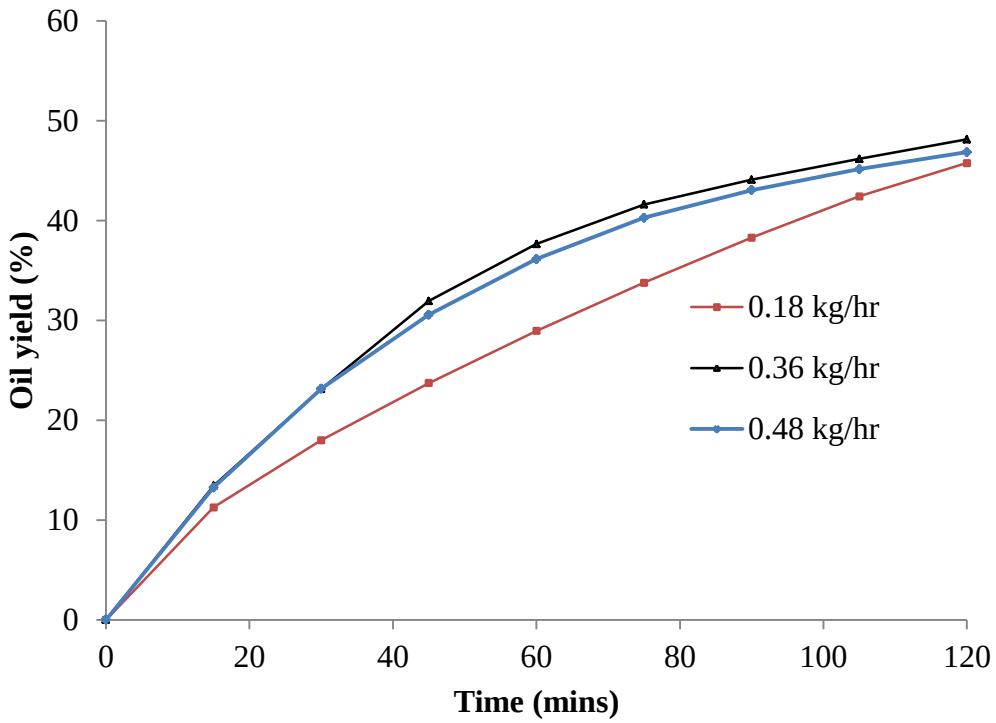

Figure 5.1: Extraction yield at P = 35 MPa and T = 313 K and solvent mass flow rate of 0.18 – 0.48 kg/hr.

5.3.2. Lipid composition

The lipid class compositions as a percentage of total lipids, for the oils extracted are summarized in Table 5.1. The major lipid classes are TAG, AMPL, PL, and FFA. TAG is the dominant lipid at 86.16 wt % in SC-CO_2 oil, 70.5 wt% in the MFM oil, and 65 wt% in the soxhlet oil. SC-CO_2 leads to higher purity oil due to the use of a non-oxidation (inert) atmosphere and mild temperatures involved, which prevent the oxidation of the polyunsaturated fatty acids [19]. SC-CO_2 is highly selective towards low polarity lipid compounds (TAG) while the co-extraction of polar impurities is limited [8,19]. This resulted in more homogeneous chemical composition in SC-CO_2 oil compared to MFM and soxhlet oils (Table 5.1). The high level of polar lipid classes in the MFM oil was

attributed to mixing of the aqueous phase (phase containing high polarity lipids) with oil at high temperature. Since phospholipids are soluble in oil at high temperature [13], polar lipids dissolved in the oil. In the soxhlet extraction method, the sample is repeatedly brought into contact with fresh solvent ensuring complete extraction [8] leading to the recovery of all lipids (polar and nonpolar lipids).

The highest FFA (8.43 ± 0.95 wt %) was measured in MFM derived oil, followed by soxhlet (7.60 ± 0.06 wt %) and the SC-CO_2 methods (2.17 ± 0.94 wt %). The hydrolysis of TAG in presence of excess water and high temperature in the MFM process may lead to high FFA content [13,34]. The FFA is produced due to hydrolysis of TAG. The oxidation of PUFA led to hydro-peroxides, secondary (aldehydes, etc.) and tertiary (acids, etc.) oxidation products. The low FFA yield by SC-CO_2 could be due to extraction conditions (i.e. inert atmosphere, low moisture, and mild temperature), which prevent the oxidation and/or hydrolysis of the TAG containing polyunsaturated fatty acids. This impacts further processing the oil to a biodiesel, as high FFA (>5%) require pre-treatment prior to transesterification [18].

Table 5.1: Lipid composition in extracted oil as a function of extraction method

% Lipid Composition	SC-CO_2	MFM	Soxhlet
Hydrocarbons (HC)	1.78 ± 1.12	0.25 ± 0.04	2.63 ± 0.80
Ethyl Esters (EE)	1.98 ± 1.14	0.00 ± 0.00	0.00 ± 0.00
Triacylglycerols (TAG)	86.16 ± 6.73	70.48 ± 2.09	65.64 ± 5.72
Free Fatty Acids (FFA)	2.17 ± 0.94	8.43 ± 0.95	7.60 ± 0.06
Sterols (ST)	0.00 ± 0.00	0.00 ± 0.00	1.17 ± 1.64
AMP- Lipids (AMPL)	3.98 ± 0.22	10.43 ± 0.22	4.11 ± 0.36
Phospholipids (PL)	3.41 ± 0.14	7.92 ± 1.70	18.87 ± 5.18
Others	1.70 ± 0.03	2.74 ± 0.01	0.09 ± 0.06

5.3.2.1 Effect of lipid compositions oil quality

The TAG lipid class is the main constituent and the most important compound for biofuels. TAG can be converted to biodiesel (fatty acid methyl esters/FAME). Sidibe et al. reported that the nature of fatty acids in TAG largely determines the oil's ability to combust properly in an engine [16]. High polar lipids (AMPL +PL) are generally undesirable in the oil due to reactivity [16,26]. Phospholipids are component of biomass cell walls/membranes and their concentration in oil largely depends on the extraction methods [13,16].

The degradation of TAG, as a result of extraction or storage conditions, give rise to increased FFA, which in turn lead to higher oil acidity [13,18,34]. Oil acidity is responsible for damage to engine delivery lines, further degradation to fuel oil and

corrosion. The SC-CO_2 method recovered the highest TAG content and minimal amounts of PL, AMPL, and FFA. As such, this oil would likely have fewer performance problems when used in conventional boilers/engines. In addition, in conversion to biodiesel, the TAG should be maximized to maximize FAME.

5.3.3. Fatty acid composition

Fatty acid compositions of lipids are summarized in Table 5.2. The major fatty extracted by each methods were palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1n-9), vaccenic acid (C18:1n-7), linoleic acid (C18:2n-6), EPA (C20:5n-3) and DHA (C22:6n-3). The fatty acid class compositions were in good agreement with composition for farmed Atlantic salmon effluents reported by Jayasinghe and Hawboldt [35], and there was no significant compositional difference between methods ($P > 0.05$).

The degree of saturated fatty acids (SFA) versus unsaturated fatty acids determined oil specific qualities. For example, Sidibe et al. [16] determined saturated oils (high SFA content) show better combustion properties such as shorter evaporation time, shorter ignition delay, and fewer deposits than the unsaturated oils and combustion quality increases with degree of saturation. However, saturated oils are more viscous at elevated temperatures than the unsaturated counterpart [8,16,36,37], which could result in poor flow properties.

Unsaturated fatty acids have a higher tendency to oxidize during storage resulting in impurities and poor combustion quality. Polyunsaturated fatty acids (PUFA) can polymerize and/or oxidize into gums, sediments or other deposits. The double bonds in

PUFA make oils more liquid than saturated fatty acids and lowers the oil melting temperature [8,16,36].

Table 5.2: Fatty acid compositions of oils extracted oil as a function of extraction methods

	This work (Salmon oil)			[20]	[20]
Fatty acids	SC-CO_2	MFM	Soxhlet	SC-CO_2	Hexanes
14:0	1.99 ± 0.01	2.68 ± 0.06	2.09 ± 0.11	4.28	3.66
16:0	14.65 ± 0.08	14.02 ± 0.77	14.88 ± 0.09	11.73	10.98
16:1n-7	5.52 ± 0.04	6.92 ± 0.11	5.63 ± 0.14	0.06	0.33
18:0	4.05 ± 0.01	4.07 ± 0.13	3.81 ± 0.14	2.93	2.04
18:1n-9	35.26 ± 0.12	31.44 ± 1.64	35.35 ± 1.49	4.62	4.38
18:1n-7	2.87 ±0 .04	3.48 ± 0.06	2.36 ± 0.28	0.83	0.78
18:2n-6	16.14 ± 0.04	15.36 ± 0.51	15.81 ± 0.28	13.55	13.61
18:3n-6	0.55 ± 0.01	0.26 ± 0.05	0.55 ± 0.03		
18:3n-3	1.82 ± 0.01	1.22 ± 0.06	1.80 ± 0.11	1.59	1.72
18:4n-3	0.66 ± 0.01	0.56 ± 0.09	0.64 ± 0.06		
20:1n-9	1.98 ± 0.01	2.11 ± 0.25	1.98 ± 0.14	0.04	0.12
20:2n-6	0.85 ± 0.01	0.77 ± 0.05	0.80 ± 0.01		
20:3n-6	0.70 ± 0.12	0.37 ± 0.05	0.60 ± 0.01		
20:4n-6	0.60 ± 0.01	0.72 ± 0.05	0.61 ± 0.05	2.15	3.22
20:5n-3	2.98 ± 0.02	4.52 ± 0.73	2.82 ± 0.25	11.91	12.22
22:1n-11	1.03 ± 0.02	1.42 ± 0.29	1.09 ± 0.12		
22:1n-9	0.27 ± 0.02	0.29 ± 0.06	0.22 ± 0.03		
22:4n-6	0.08 ± 0.07	0.16 ± 0.02	0.19 ± 0.07	2.15	3.22
22:5n-3	1.15 ± 0.01	1.66 ± 0.09	1.16 ± 0.12	2.01	2.21
22:6n-3	2.98 ± 0.02	3.49 ± 0.67	2.94 ± 0.30	13.15	13.86
24:1	0.12 ± 0.01	0.20 ± 0.02	0.25 ± 0.02		
Sums	96.22 ± 0.68	95.18 ± 5.82	95.58 ± 3.88		
Other	3.78 ± 0.08	5.81 ± 0.43	4.42 ± 0.17		
SFA	21.20 ± 0.08	21.43 ± 0.86	19.96 ± 2.31	21.09	18.87
MUFA	48.14 ± 0.10	47.36 ± 1.95	49.78 ± 3.72	7.70	7.80
PUFA	30.33 ± 0.17	31.71 ± 1.31	29.88 ± 1.41	56.32	59.59
P/S ratio	1.43	1.48	1.50		

SFA-saturated fatty acid; MUFA-monounsaturated fatty acid; PUFA-polyunsaturated fatty acid; MFM – Modified fishmeal method, P/S – ration of PUFA to SFA.

5.3.4. Thermal analysis (TGA)

TGA analyzes the thermal stability by relating oil physicochemical properties to weight changes as a function of temperature [38]. The thermal behavior of salmon oil was studied under nitrogen atmosphere between 0 °C and 800 °C (Fig.5.3). The onset weight loss temperature of fish oils from both methods starts at approximately 225 °C which is in agreement with 220 °C reported by Araujo et al. [39]. Between 367 °C and 497°C, the rate of weight loss increased rapidly with temperature. The MFM oil shows some resistance to thermal degradation compared SC-CO_2 oil, this is due to impurities/undesired products levels in the MFM oil compared to the SC-CO_2 oil. Sathivel et al., Wiedermann and Chiou et al. [30,40,41], proposed the impurities in crude fish or vegetable oils absorb heat, slowing the decomposition of the oil [30,40]. The total weight loss between 367 °C and 497 °C is approximately 90 wt. %. After 500 °C, 0.07 and 0.36 wt. % recalcitrant materials remained as residue in the SC-CO_2 and MFM oil respectively, verifying the higher purity of the SC-CO_2 compared to the MFM oil. Thermal polymerization reactions in edible oils occur in temperature between 200 °C and 300 °C in inert or nitrogen environment [39,42] but was not detected in this work (Fig.5.3).

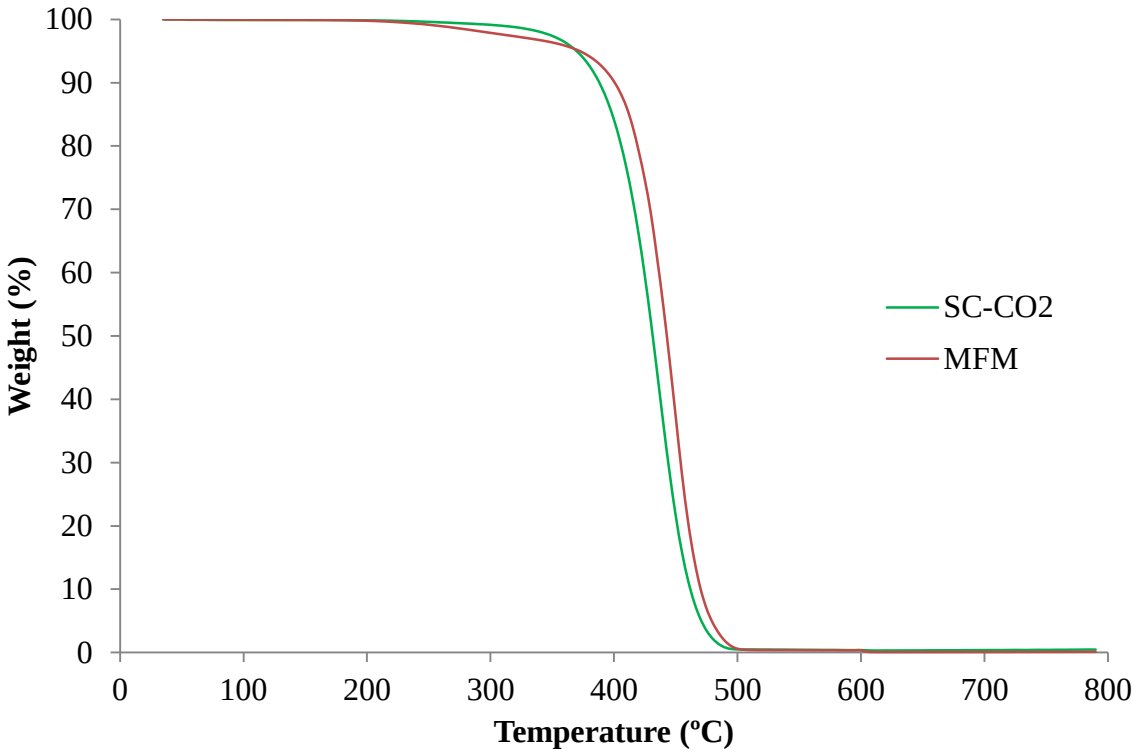

Figure 5.3: Thermal degradation of the crude Salmon oil recovered by SC-CO_2 and MFM methods in nitrogen atmosphere.

5.3.5. Calorific value/High heating value (HHV)

The high heating values (HHVs) of the oil are outlined in Table 5.3. The HHV are approximately 40, 39 and 38 MJ/kg for the SC-CO_2, soxhlet, and MFM extracted oil respectively. The difference in HHV of the SC-CO_2 and MFM oil may be due to lower moisture content of SC-CO_2 oil. The HHV of the salmon oil is in the range of seed oil based biodiesel (39 MJ/kg to 43.33 MJ/kg) reported by Sivaramakrishnan et al. [43] and lower than that of petroleum diesel (49.65 MJ/kg). The values from this study are also very close to HHV for vegetable oils between 36 and 40 MJ/kg reported by Blin et al. [13].

Table 5.3: Some salmon oil properties as function of extraction methods

Method	SC-CO_2	MFM	Soxhlet
Recovery (%)	48.14 ± 1.06	20.70 ± 2.76	52.84 ± 1.26
HHV (MJ/kg)	39.58 ± 0.03	38.41 ± 0.10	39.49 ± 0.12
FFA (%)	0.83 ± 0.05	7.32 ± 1.9	
Peroxide Value	4.34 ± 0.56	5.60 ± 0.38	
Sediment (%)	0.00	0.95 ± 0.1	
Free water (%)	0.00	1.02 ± 0.1	
Moisture (%)	0.134 ± 0.002	0.24 ± 0.02	0.005 ± 0.003
Temperature (℃)	Kinematic viscosity (cSt)		
20	6.72 ± 0.03	5.90 ± 0.04	
40	3.22 ± 0.00	2.53 ± 0.02	
60	1.80 ± 0.00	1.31 ± 0.02	
	Density (kg/m^3)		
20	919	996	
40	905	991	
60	892	985	

5.3.6. Melting point

The DSC thermograms (Fig. 5.4) show two broad endothermic peaks for the crude salmon oil extracted using SC-CO_2 and MFM processes. The melting point of salmon oil in both methods ranged from -48.10 ± 2.21 to 17.25 ± 1.87 °C and both freeze at approximately -12.36 °C. The negative melting points of the oils were attributed to the

presence of triacylglycerol which contain unsaturated fatty acids [30,31,36]. The higher degree of unsaturated fatty acids in fish oil results in melting at lower temperatures [31]. The thermogram peaks for the oils were not sharp due to the presence of impurities such as phospholipids, free fatty acids, aldehydes, particulate matters, ketones, water, and pigments. These impurities result in a melting behavior typical of pure fatty acids [31,36]. The similarity in melting points of oils from both methods is a reflection of their comparable fatty acid composition (i.e. similar proportions of SFA, MUFA, and PUFA between the oils from the three methods) (Table 5.2).

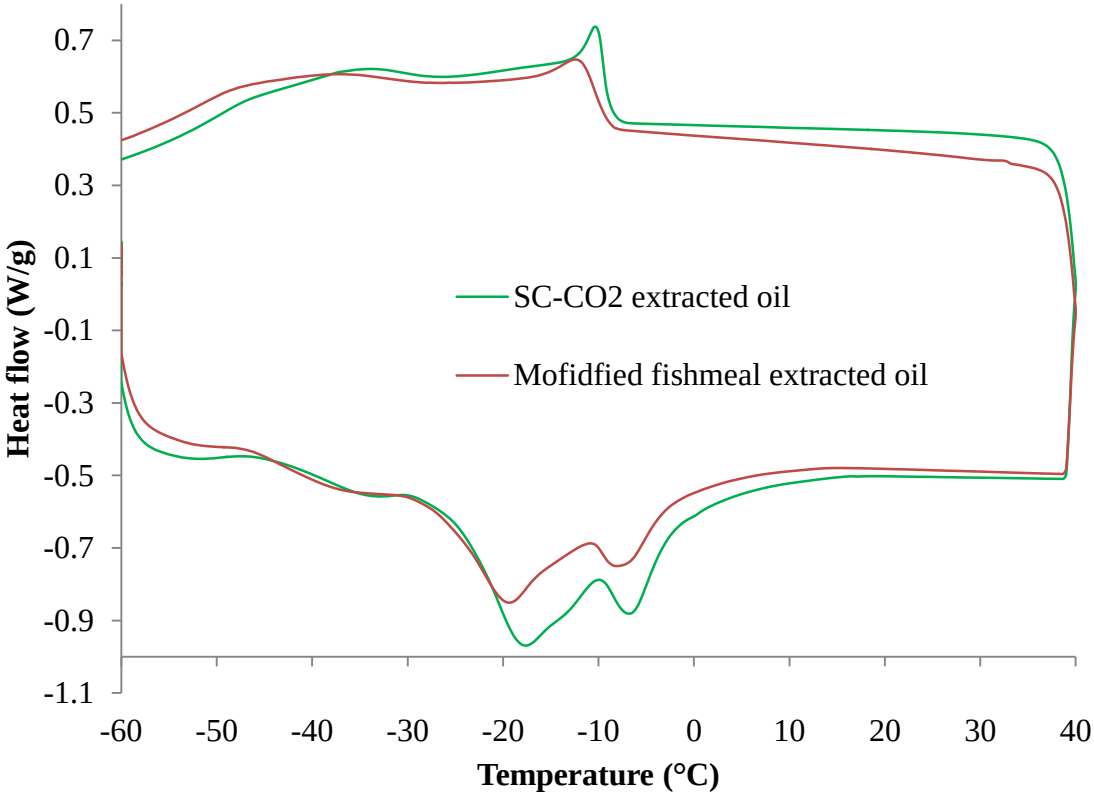

Figure 5.4: DCS thermograph of the crude salmon oil extracted using SC-CO_2 and MFM methods.

5.3.7. Rheological properties

The apparent viscosity as a function of shear rate and temperature was investigated (Fig. 5.5). At 60 ^{o}C the MFM derived oil viscosity decreased from 65.1 cP to approximately 17.1 cP as shear rate was increased from 13 s^{-1} to 172 s^{-1} and remained constant after. The same trend was observed for MFM derived oil at 40 ℃ and 20 ℃ (Fig.5.5). At 60 ^{o}C, the shear rate had little impact on the SC-CO_2 oil viscosity. A similar trend was observed at 40 ℃ and 20 ℃. The decrease in viscosity with increasing shear rate indicates that the MFM oil exhibited shear-thinning non-Newtonian behavior which is in agreement with our previous work [26]. Sathivel et al. [44] determined crude fish oils contain soluble impurities such as phospholipids, FFA, peroxides and their degraded products that are highly interactive with oils and lead to formation of an aggregated colloidal, which often shows shear-thinning behavior [44]. In the case of SC-CO_2 extracted salmon oil, the viscosity is almost constant at all temperatures studied indicating Newtonian behavior. Sathivel et al. [44] showed the change in catfish oil from shear-thinning Newtonian behavior (before refining) to Newtonian behavior (after refining e.g. degumming, neutralization, bleaching, and deodorization). Each refining stages removes impurities, minerals complexed by phospholipids, and other unwanted polar compounds. The SC-CO_2 oil contains less than 10 % impurities and over 85 wt % TAG (Table 5.1).

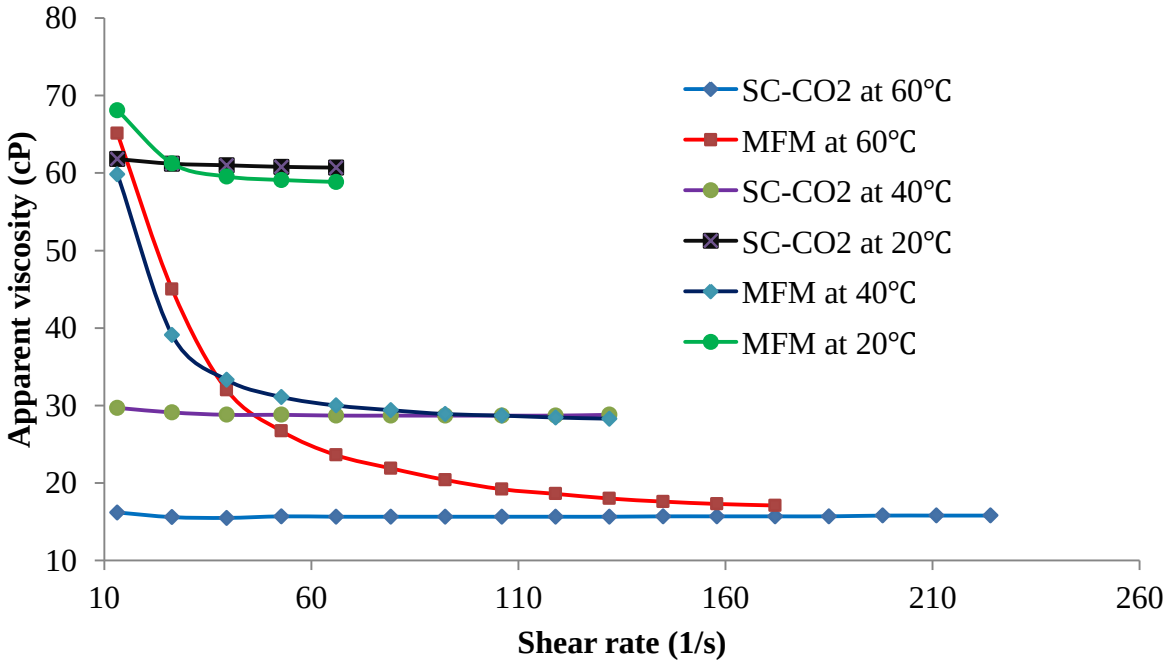

Figure 5.5: Change in apparent viscosity with shear rate at 20, 40 and 60 ^{o}C for salmon oils from MFM and SC-CO_2 methods.

Closer observation of the flow behavior index (n) shows that MFM extracted oil behaves pseudoplastically (i.e n < 1). The power law parameters were given in Table 5.4. The flow behavior index (n) ranges between 0.53 and 0.89, indicating non-Newtonian behavior [30,31]. These values are similar to those reported for unpurified salmon oil by Huang and Sathivel [31], and unrefined Pollock oil by Sathivel et al. [30]. In practice, specific additives are added to viscous material to achieve pseudoplastic flow behavior. This behavior is beneficial to fuel flow through pipes and hoses as stress forces applied through pumps reduce fuel viscosity. The stronger the shear force subjected to the pseudoplastic material, the higher the thinning effect and the higher the reduction in viscosity [45], hence, the better the flow properties. The flow index (n) of the SC-CO_2

extracted salmon oil is very close to unity (i.e. n > 0.90), indicating that the oil, like diesel fuel oil and other mineral oils, is Newtonian in nature.

Table 5.4: Flow behavior index, consistency index, and activation energy of salmon oils as a function of extraction methods

	$SC\text{-}CO_2$ oil			MFM oil		
T (℃)	Flow index (n)	K	R^2	Flow index (n)	K	R^2
20	0.9915 ± 0.00	0.6284 ± 0.01	1.0000	0.89 ± 0.01	0.04 ± 0.01	1
40	0.9914 ± 0.01	0.2980 ± 0.01	1.0000	0.69 ± 0.00	0.07 ± 0.00	0.998
60	0.9977 ± 0.00	0.1589 ± 0.00	0.9999	0.53 ± 0.01	0.28 ± 0.01	0.996

	$SC\text{-}CO_2$ oil	R^2	MFM oil	R^2
Ea (kJ/mol)	27.4 ± 0.1	0.998	31.04 ± 0.6	0.998
A (x 10^{-8})	8.21 ± 0.3	0.998	1.69 ± 0.4	0.998

In eq. (2) the activation energy, E_a, (kJ/mol) represents the energy barrier to flow [30-32,46]. The frequency factor or viscosity coefficient at a reference temperature, *A*, (Pa.s) and *Ea* values were determined. In general, the higher the activation energy, the more sensitive the viscosity will be to temperature [46]. As shown in table 4, the *Ea* for the $SC\text{-}CO_2$ oil is lower (27.40 kJ/mol) compared to the MFM oil (31 kJ/mol). This indicated that the temperature effect on viscosity is more dominant in MFM extracted oil than $SC\text{-}CO_2$ extracted oil. This property reflects the heterogeneous nature of MFM oil compared to $SC\text{-}CO_2$ oil.

5.3.8 Water and sediment, moisture, FFA, peroxide value, density and kinematic viscosity

The free water, sediment and moisture content of oil are presented in Table 5.3. Free water and sediment were not detected in the SC-CO_2 oil. Approximately 1.0 and 1.2 wt % free water and sediment respectively were detected in the MFM oil. The moisture content is lower (0.13 wt %) in the SC-CO_2 oil compared to the MFM oil (0.24 wt %). The water removal through freeze-drying is a contributing factor for the low moisture in the SC-CO_2 oil. As the SC-CO_2 process is solubility driven the resulting oil is sediment free. Generally, the MFM process largely depends on centrifuge efficiency for good separation between the oil, water and sediment. Water in the oil can hydrolyze the TAG leading to acidic oil. Water also impacts fuel filter cartridges and during combustion can cause cavitation at the piston head [13].

The SC-CO_2 oil FFA and PV levels were lower compared to the MFM oil. Lower values of FFA and PV indicate less degradation. High FFA and PV value of oil lowers its quality as a fuel oil. The oil densities at 20, 40, and 60 ^{o}C are outlined in Table 5.3. The SC-CO_2 oil density is lower than the MFM extracted oil at all temperatures studied. The higher percentage impurities in the MFM oil may be responsible for denser oil. The density of SC-CO_2 oil agrees with red (900 kg/m^3) and pink salmon (810 kg/m^3) oils [37]. Compared to petroleum fuel oil distillates, the densities are close to No.6 fuel oil (985 kg/m^3), DMC marine distillate fuel (920 kg/m^3) and marine residual fuels (960-1010 kg/m^3) [4]. The kinematic viscosity of the oil extracted from both SC-CO_2 and MFM

processes ranges between 1.3 and 6.7 cSt. These values are within diesel fuel standard ASTM D396, 2–3.6 cSt and 5.8–26.4 cSt at 38 °C for grade No. 2-D (diesel) and grade No. 4-D (medium distillate) respectively [13].

5.4 Conclusion

This study has demonstrated the effect of extraction methods on chemical composition, thermal, physicochemical, and flow properties of salmon waste derived oil. A comparison of the oil by SC-CO_2 to oil from the MFM and soxhlet methods shows that SC-CO_2 produces higher purity oil. The SC-CO_2 recovered more oil (91 wt %) from the waste compared to MFM (71wt %). There is significant difference (P<0.05) in TAG recovery between SC-CO_2 oil (86 wt %), MFM (70 wt %), and soxhlet (66 wt %) oils. The SC-CO_2 limits the extraction of high polar compounds (PL and AMPL) which are undesirable in crude bio-oil when used as a as fuel or biodiesel feedstock. In addition, the SC-CO_2 oil is lower in FFA compared to MFM and soxhlet. High FFA content in fuel oil results in high acidity that leads to corrosion and engine inefficiency and intensifies the biodiesel conversion process.

The MFM oil exhibited a shear-thinning non-Newtonian flow behavior, hence, has better flow properties than SC-CO_2 oil which exhibited Newtonian behavior. However, the flow behavior is as a result of impurities such as FFA, primary oxidation products, minerals, pigments, moisture, and phospholipids.

The SC-CO_2 oil quality would require minimal or no further refining steps for fuel use however the process is energy intensive therefore energy costs must be balanced against higher oil quality and other environmental benefits. The MFM and soxhlet oils are of lower quality due to impurities and further refining steps (such as degumming, neutralization, bleaching, and deodorization) will be required to improve the oil's quality leading to additional cost. This will be of particular concern when for any upgrading to

biodiesel, as impurities in the oils require pretreatment prior to the transesterification process.

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CHAPTER 6

LIFE CYCLE ANANLYSIS (LCA) COMPARISON OF FISH OIL EXTRACTION METHODS

6.1 Introduction

In the area of sustainable energy substitutes, biofuel has been proposed as a replacement for fossil fuels. A number of biomass sources have been proposed as feedstock for biofuels and a brief summary is outlined in Table 6.1.

Table 6.1: Studies on alternative options for petroleum fuels

Feedstock	Study	Reference
Salmon oil	Biodiesel production	[9]
Fish oil and fish oil biodiesel	Comparative study with mineral fuel	[10]
Fish oil biodiesel	Performance and emission evaluation	[11]
Vegetable oil	Biodiesel production	[12]
Fish oil	Alternative Fuel for Conventional Combustors	[13]
Fish oil	Low-Grade Fuel	[14]
Marine oils	Biodiesel/marine diesel blends on stationary diesel engine	[15]
Soap stock of marine fish	Fuel properties of biodiesel	[16]
Marine fish wastes	Engine performance and emission characteristics of fish-oil biodiesel	[17]
Waste cooking oil	Biodiesel Production	[18]
Rubber seed oil	Biodiesel production from	[19]
Waste fish oil	Renewable fuel in Iran	[20]

The quality of the biofuel are influenced by the quality of the feedstock, the method of extraction [1-3] and the processing conditions, which need to be carefully managed to obtain a high quality fuel. Atabani et al. [4] showed that biodiesel quality is a function of feedstock fatty acid composition, production, and refining method(s). Biofuels from fish waste has been proposed as a possible low quality fuel oil or as a source for biodiesel

production [9, 10, 14, 15 and 20]. Fish oil is also extracted for edible oils. In remote locations or fish plants with limited infrastructure the extraction of edible oils may not be possible due to distance to market, regulatory requirements for storage and transport, among other factors. Fish oil extraction methods can be physical, biological and chemical and various methods are summarized in a previous review [3]. Physical extraction, also known as wet reduction, is the most widely used fish oil recovery process and includes basic unit operations such as grinding, heating, pressing, and separation [3,5]. The enzymes are employed to accelerate the process of breaking the proteins into smaller groups, enhancing oil extraction in biological extraction, while solvent extraction uses chemicals or solvents for oil extraction. Supercritical fluid extraction (SFE) process using carbon dioxide (SC-CO_2) is advantageous compared to conventional extraction processes due to high quality of oils, reduced environmental impacts, mild extracting temperature, etc. This method minimizes the oxidation of the oil (which reduces fuel quality and ability to refine to biodiesel) and extracts low polar lipid substances [6]. However, an environmental assessment of this process for extraction of oil for fuel from fish waste relative to conventional methods has not been done.

Life cycle assessments (LCA) are an environmental assessment tool used in the quantification of environmental burdens and potential impacts associated with the whole life cycle of a product, process, or activity [7,8]. A LCA can assess the efficiency, environmental impacts and cost benefits of products and processes. Many researchers have studied LCA of biofuel products and processes (Table 6.2), however, the LCA on oil extraction/recovery processes is less well studied. The purpose of this work is to carry

out the LCA of different extraction processes (modified fishmeal process (MFM), SC-CO_2, and soxhlet extraction methods) of fish oil from processing plant waste. The material and energy requirements from each process during extraction were estimated using a process simulator (Aspen Plus®) and published data and input into SimaPro 7 for LCA.

Table 6.2: Some studies on LCA of biofuel and biodiesel processes

Study	Reference
LCA of the biofuel production process from sunflower oil, rapeseed oil and soybean oil	[21]
LCA of biodiesel production from waste cooking oil	[22]
LCA on microalgae biodiesel production	[23]
LC cost and sensitivity analysis of palm biodiesel production	[24]
LCA of Jatropha biodiesel production	[8]
LCA of soybean biodiesel production	[25]
LCA of palm biodiesel: Revealing facts and benefits for sustainability	[26]
LCA energy, environment and economic assessment of soybean-based biodiesel as an alternative automotive fuel in China	[27]
LCA studies comparing biodiesel synthesized by conventional and supercritical methanol methods	[28]
Carbon cycle for rapeseed oil biodiesel fuels	[29]
Life cycle model of alternative fuel vehicles: emissions, energy, and cost trade-offs	[30]

6.2 Scope, purpose and system boundary.

The scope of the LCA includes the energy consumption and material emissions (Fig.6.1) of the proposed fish oil extraction processes. Fish waste can vary; in the study we considered a comprehensive waste composition (heads, guts, trimmings, and frames). The crude fish oil extracted is the final product. As the study is focused on comparing extraction methods, the emissions associated with fish oil as a fuel were not included; however the quality of the oil for fuel use as a function extraction method will be evaluated. The LCA of un-recycled fish waste has been previously studied in our lab [34] therefore, not included in the current research. The process boundary included the materials, energy, and process effluents (Fig.6.1). The functional unit used for inventory analysis is per 1 kg of crude fish oil (product).

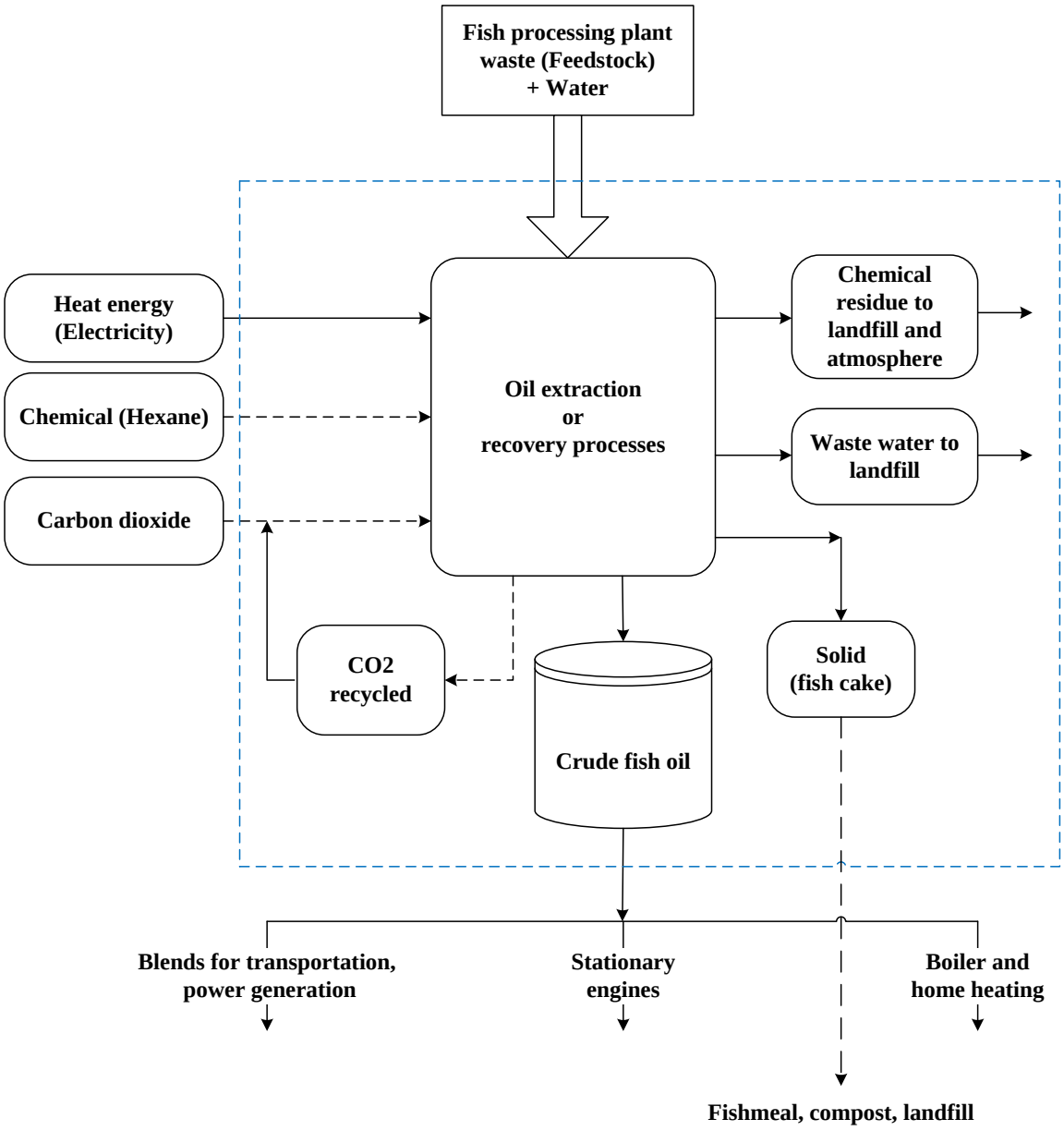

Figure 6.1: LCA process boundary

6.2.1 Methodology

LCAs are used to link life cycle inventory (LCI) results to environmental impacts. In Sigma Pro the method used to link LCI results is "IMPACT 2002+". In this method, the LCI results are first linked to 14 midpoint categories, which are in turn linked to four

damage categories. The term “midpoint” is used to indicate that in the overall impact pathway, the midpoint represents an intermediate point between the LCI results and the end point (damages). The 14 categories are outlined in Figure 6.2 [31]. The damage categories take the midpoint categories and further classify them into an impact on the environment or damage. The Impact 2002 method further converts the qualitative damage to a quantified damage. The quantification is done by multiplying the damage factor (characterization factor) with the inventory data [31].

The inventories through the midpoint category are expressed in unit of a reference substance related to the four damage categories. The potential environmental impacts associated with the input and output of a process/product is evaluated via the use of characterization factors (CFs).

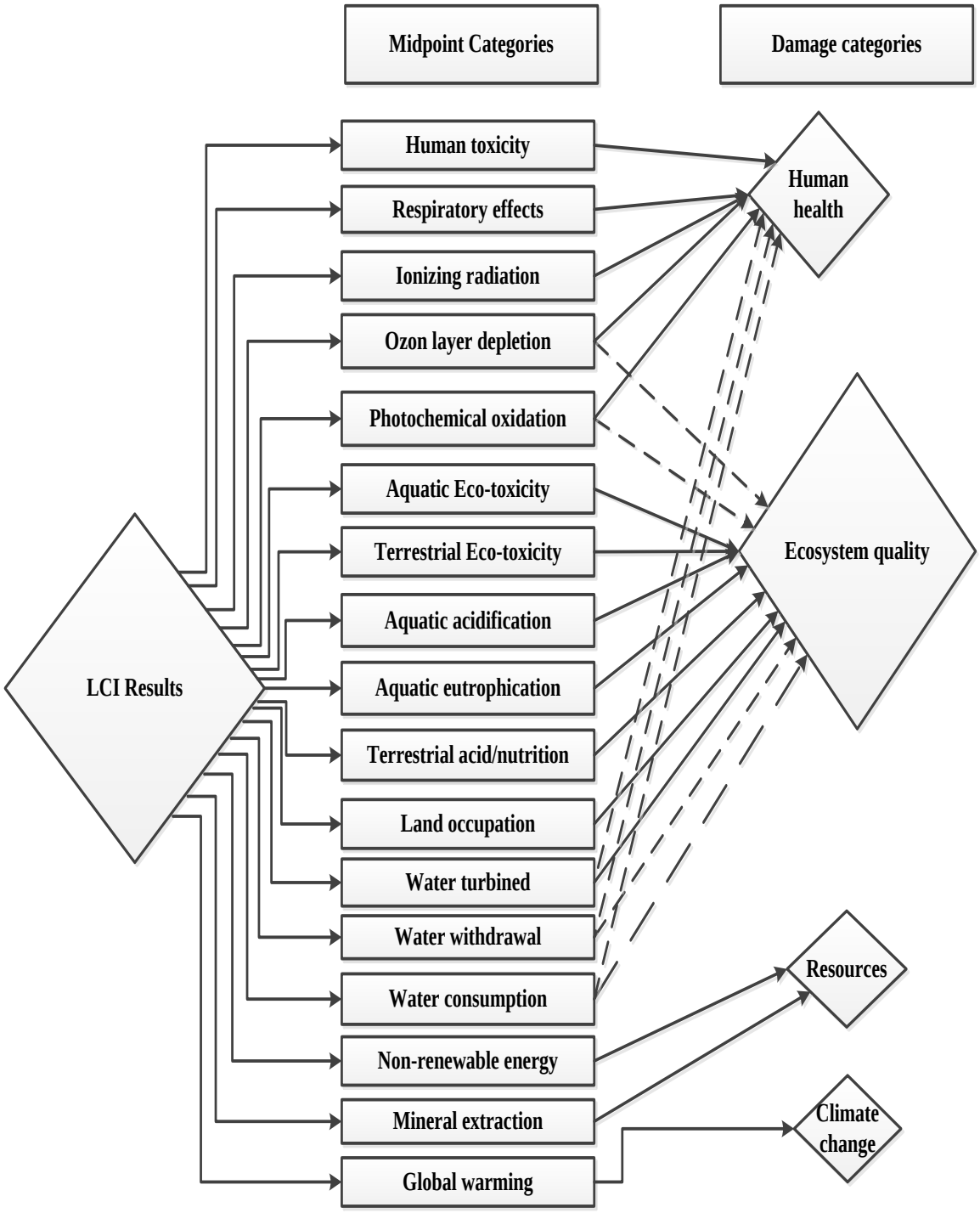

Figure 6.2: Impact 2002 + LCA methodology

6.2.2 Life Cycle Inventory (LCI)

The LCI was generated through the energy and material consumed during extraction processes (Tables 6.3a and 6.3b).

Table 6.3a: Material (kg) inventories of extraction processes

Material /energy	Methods		
	$SC\text{-}CO_2$	Soxhlet	MFM
Oil out (kg)	1.00	1.00	1.00
Fish-waste in (kg)	2.25	2.10	4.88
Fishcake out (kg)	1.26	1.25	1.44
CO_2 in (kg/h)			
CO_2 out (recovered)(kg/h)			
Hexane in (kg)		33.24	
Hexane out air (kg)		1.66	
Hexane recovered (kg)		31.58	
Water out (kg)			1.95
Organic matter out (kg)			0.49
Freeze dry process			
Fish-waste in (kg)	4.50	4.20	
Fish-waste out (kg)	2.25	2.10	
Water out (kg)	2.25	2.10	

Table 6.3b: Energy (kJ) inventories of extraction processes

Equipment	MFM	Soxhlet	SC-CO2
Conveyor	486.30	187.53	203.94
Grinder/Crusher	482.44	186.04	202.32
Pump	486.75	187.71	204.13
Heat exchanger	5582.46	2152.78	2341.15
Pump	486.75		
Twin screw press	573.53		
Vertical centrifuge	1.51		
Freeze dryer		1202.99	1308.25
Conveyor		187.53	203.94
Sub-Cooler		221.21	
Rotaryvap/Evaporator		20.78	
Grinder		186.04	202.32
CO_2 Recovery			12958.18
Total energy input (MJ)	8.10	4.53	17.62

The mass of oil produced per mass of fish waste, chemicals required, and associated waste streams for each process were determined from lab and pilot scale experiments (Section 3.1 of chapter 5). The process flow diagram for each process is outlined in Figures 6.3a – 6.3c and is based on lab and commercial scale data.

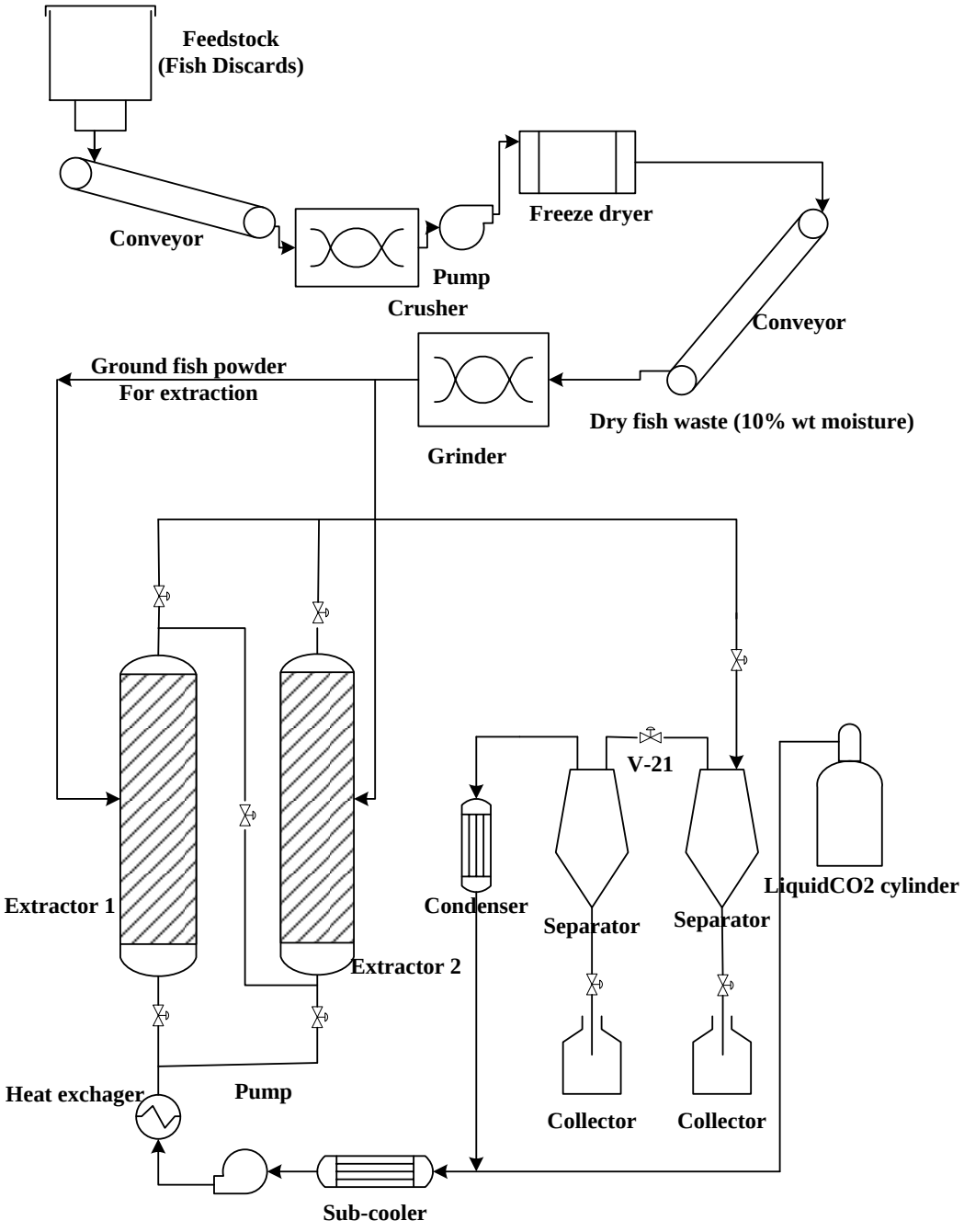

Figure 6.3a: SC-CO_2 extraction process flow diagram

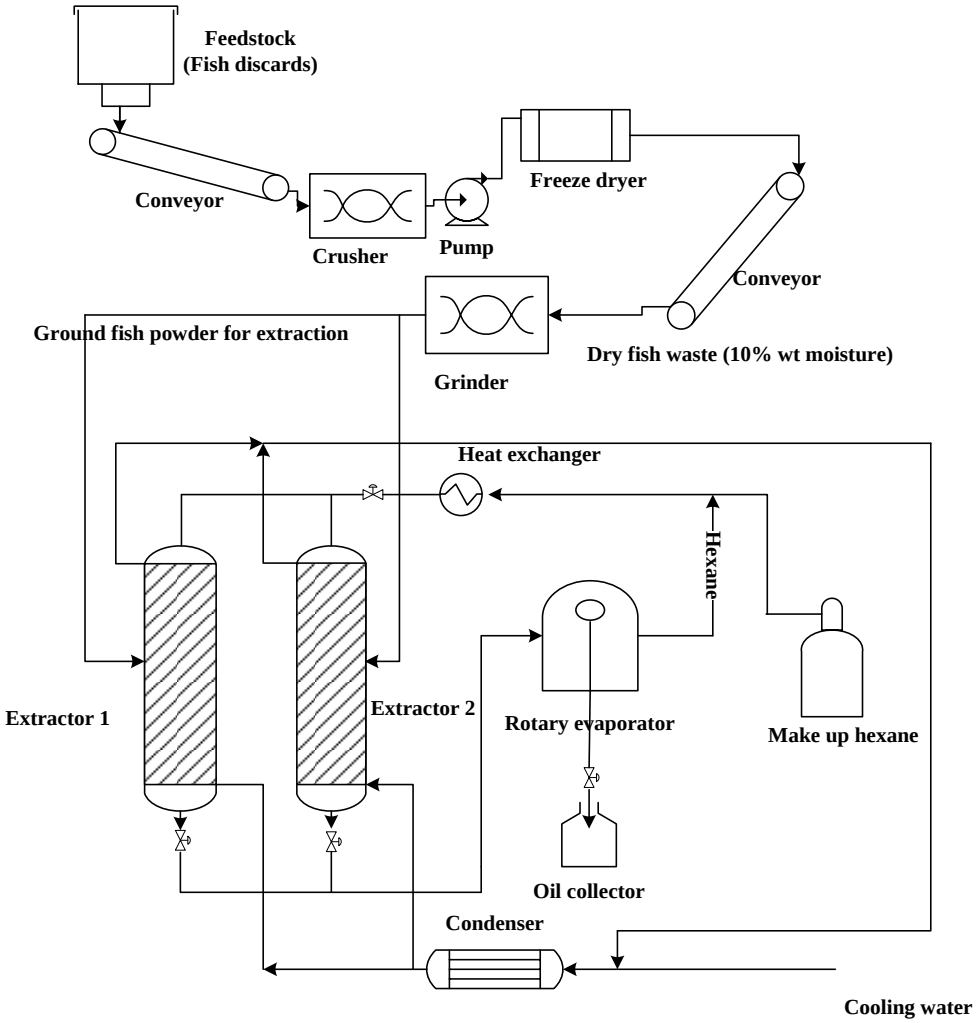

Figure 6.3b: Soxhlet extraction process flow diagram

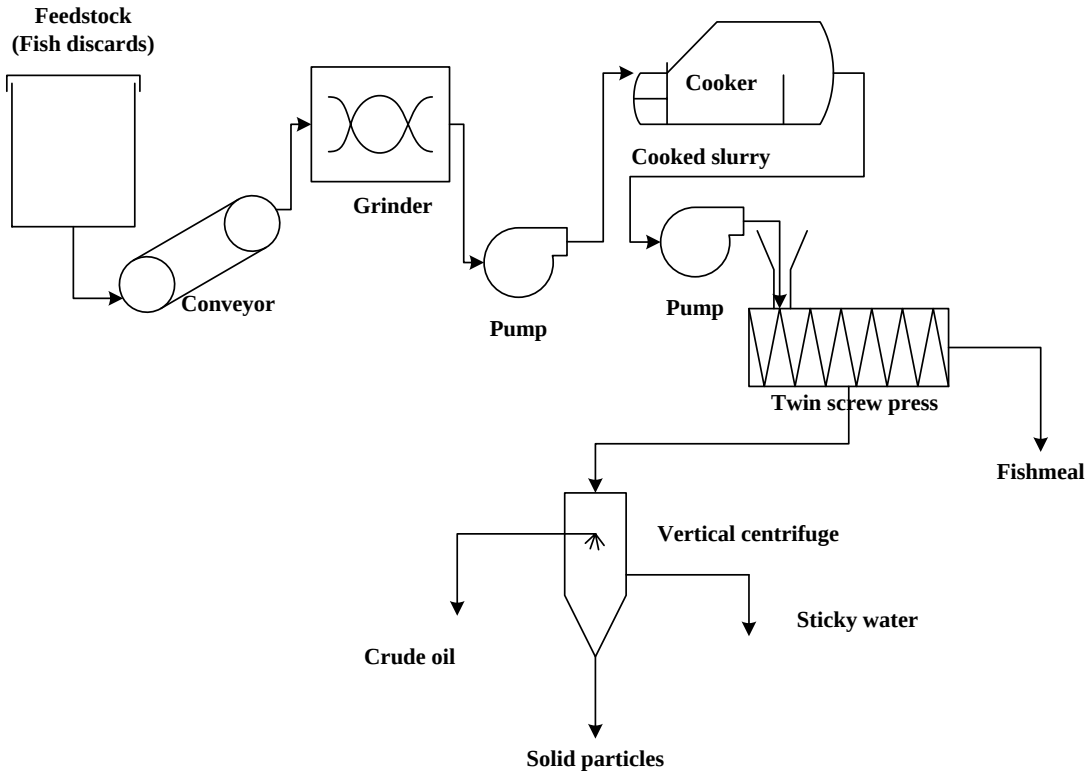

Figure 6.3c: MFM extraction process flow diagram

The energy required for each unit operation, such as pumps, heat exchangers, freeze dryers, sub-coolers and vacuum evaporators, were modeled using Aspen Plus®. The energy input to conveyors, grinder/crusher, screw press and vertical centrifuge were estimated using energy equations, while, the energy consumed in CO_2 compression and recovery was estimated using the energy analysis of supercritical carbon dioxide extraction processes from Sievers and Eggers [32], and Smith et al. [33]. The results were exported to SimaPro®7 to obtain the environmental impact assessment from the use of energy and resources in each extraction process.

6.2.3 Materials and experimental methods

The feedstock is a by-product of the fish industry; specifically the offcuts (offal) from farmed salmon (*Salmo salar Linnaeus*), from Cooke aquaculture provided by the Centre for Aquaculture and Seafood Development (CASD). The detailed description of materials and experimental methods for the fishmeal process can be found in Section 2.1 of chapter 3, soxhlet extraction method in section 3.0 of chapter 4 and the SC-CO_2 in section 3.2 of chapter 4.

6.3 Environmental impact assessment

6.3.1. Characterization

Figure 6.4 summarizes the damage characterization as a function of process. Among all the extraction processes, only the soxhlet extraction has an impact of over 95% in the category of carcinogens, ozone layer depletion, respiratory organics, terrestrial ecotoxicity, and mineral extraction. The soxhlet extraction process in general has a high impact due to emissions of hexane both in air and water or soil. The MFM process has an impact of 92% on the aquatic eutrophication and 75% on aquatic acidification due to process water discharge into the ocean and water bodies. This effluent is rich in nitrogen compounds and other organics.

In the categories of aquatic ecotoxicicity, terrestrial acid/nutri and non-renewable energy, the soxhlet extraction method has a contribution that is relatively high compared to SC-CO2 and MFM methods. This impact can also be attributed to the impact of air emissions of volatile hydrocarbons (hexane) during the extraction process.

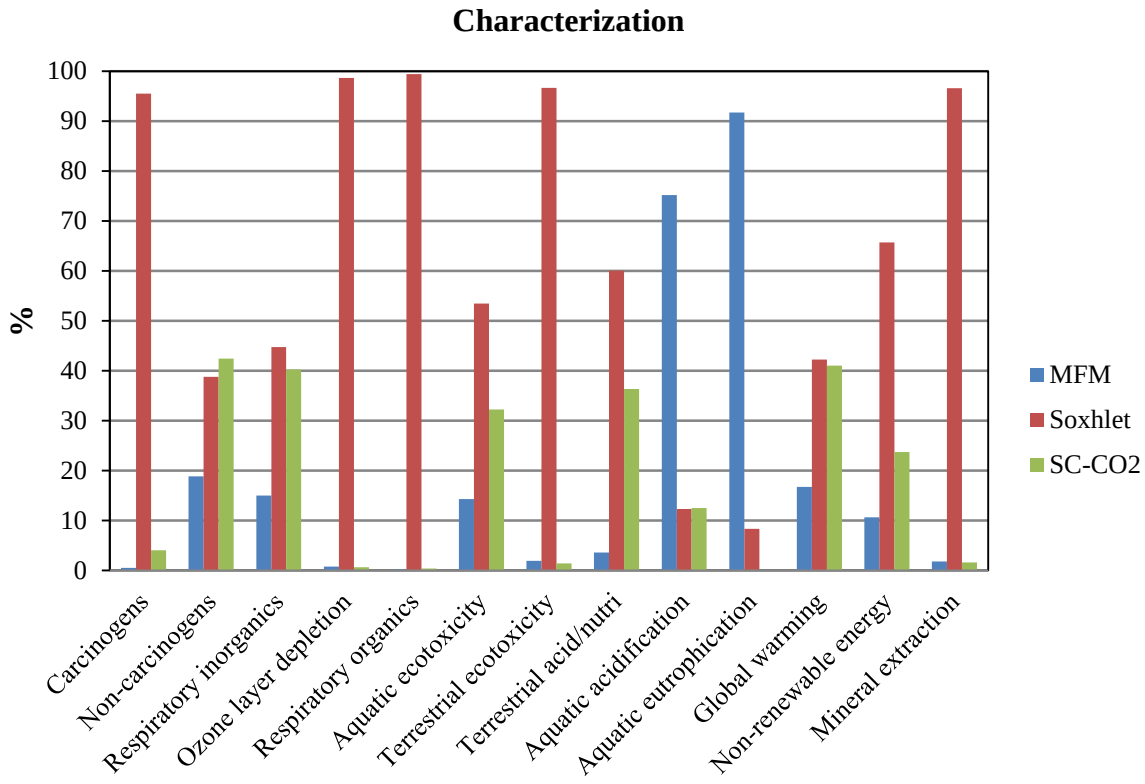

Figure 4: Environmental impact characterization of the LCA of extraction processes.

6.3.2. Damage assessment

Figure 6.5 outlines the impact assessment, which refers to the categories as 'harm' instead of the 'impact' (as with the characterization). In the category of human health (carcinogens and non-carcinogens combined) the soxhlet method contributed the highest damage which, in this category, would be attributed to human exposure to n-hexane during the process. Hexane is toxic and the most probable route of human exposure to hexane is by inhalation and via the skin. The damage caused by the SC-CO_2 in the human health category is 35% which is mostly emissions due to energy usage (fuel, electricity etc.). The damage contribution of the MFM process in this category is the least (13%) which is also as a result of emissions from energy usage. Similar damage trends (soxhlet

> SC-CO_2 > MFM) were observed with the ecosystem quality, climate change, and resources. The high damage contributions from the soxhlet method would be attributed to the damages effect from both energy consumption and solvent residue. However, the higher effect from the SC-CO_2 compared to MFM process is a reflection of higher energy required (17.62 MJ) to produce 1 kg fish oil in SC-CO_2 than (8.10 MJ) in MFM process (Table 6.3b).

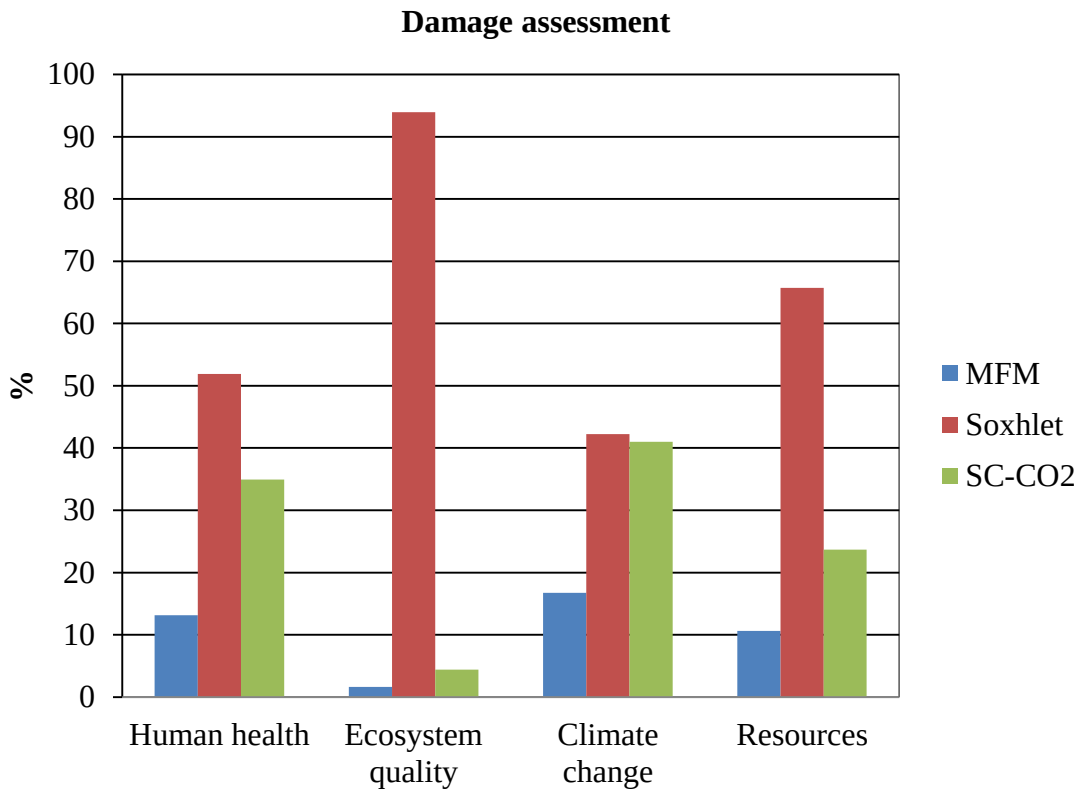

Figure 6.5: Environmental impacts by categories of damages for all extraction methods.

6.3.3. Normalization.

The objective of normalization is to reflect the relative magnitude of each impact category of a product or system [31]. Normalization also assesses the impact category contribution as a global environmental problem [21]. Again, the soxhlet extraction

method has the highest impact in all categories (human health, ecosystem quality, climate change and resources) (Fig.6.6). The overall analysis shows that the soxhlet method uses a large amount of resources: chemical, fuel, electricity, etc. The production, transportation and storage stages of hexane is possibly the highest contributor as the soxhlet method uses the least energy, 4.53 MJ (Table 6.3b).

Comparing the SC-CO_2 and MFM process (Fig.6.6), the SC-CO_2 contributed higher impacts in all categories (human health, ecosystem quality, climate change and resources) than the MFM extraction process, and the impacts by both methods are apparently due to energy consumptions.

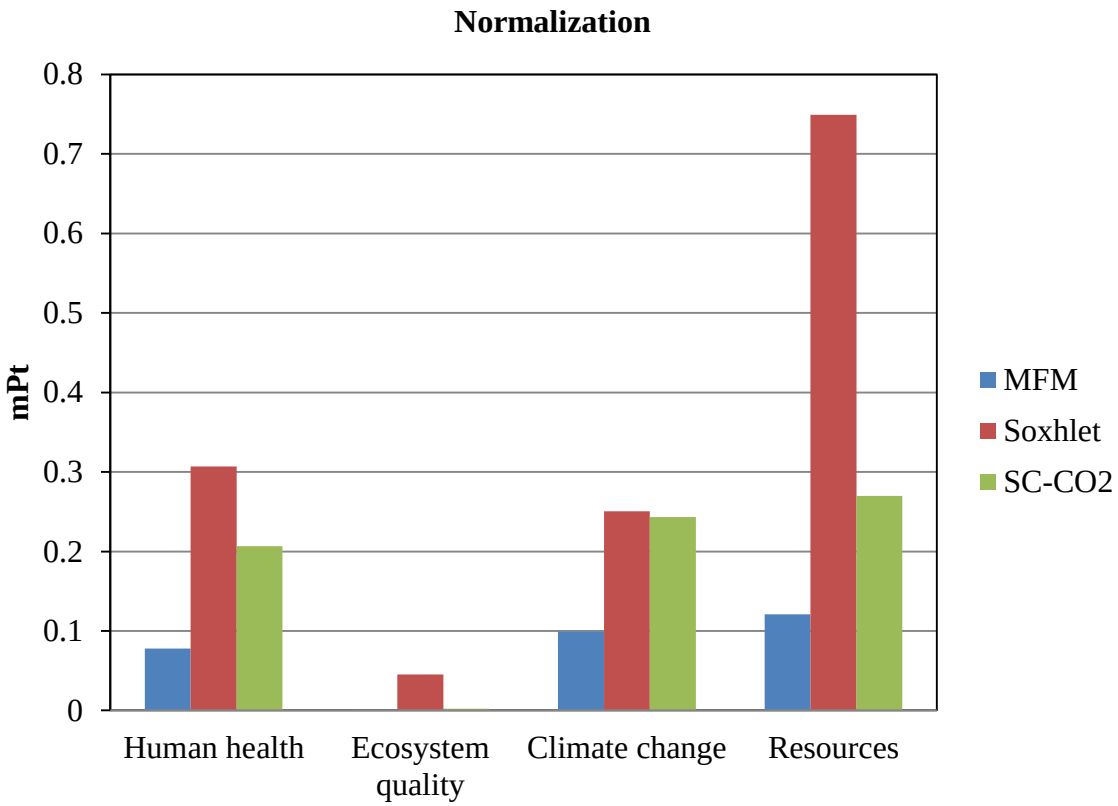

Figure 6: Normalization of impact categories as function of extraction methods.

6.3.4. Single score

When comparing the impact assessment in terms of single score method, the normalized and the weighted values of all impact categories were added up (Fig.6.7). According to the analysis, the soxhlet extraction method has the highest overall environmental impact/score (1.352045 mPt), the SC-CO_2 has the second highest (0.722109 mPt) and the MFM has the least (0.297142 mPt) (Fig.6.7). The analysis indicates that the oil extraction using soxhlet uses large amount of resources (55.41%) such as solvent, fuel, electricity, etc. Also, the impact due to human health is relatively high (22.70%) and that of climate change is 18.54%. In the case of the total environmental impact of SC-CO_2 method, the impact due to the human health, climate change and resources are 28.61, 37.39 and 33.71% respectively. The impact due to the ecosystem quality is negligible (< 1%). The most probable cause of environmental impact in the SC-CO_2 process is due to resources use (fuel, electricity, etc.).

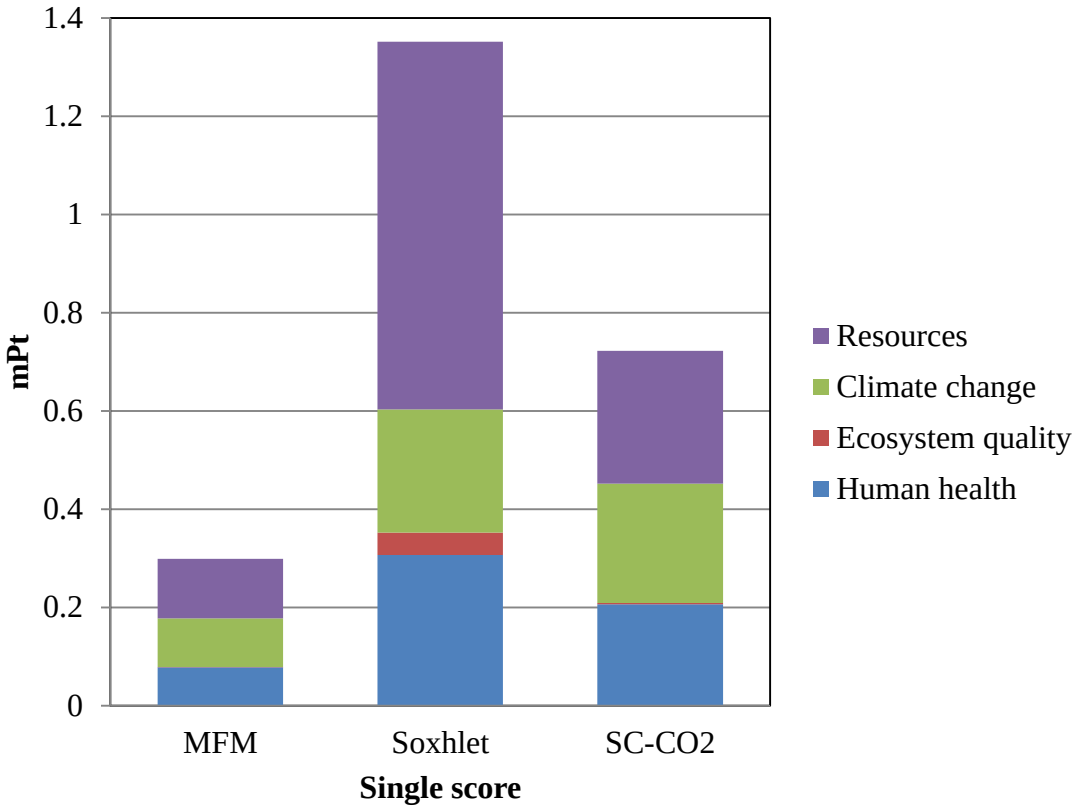

Figure 6.7: Single score of damage assessment for all extraction methods.

6.3.5 Quality assessment

Table 6.4a summarized the quality of oil recovered as a function of extraction methods. The triacyglyceride (TAG) is the dominant lipid class and most important component of the oil. The SC-CO_2 method has the highest TAG recovery (86 wt %), compare to the MFM (70wt%) and the soxhlet method (65 wt%). SC-CO_2 leads to higher purity oil due to the use of a non-oxidation (inert) atmosphere and mild temperatures involved, which prevent the oxidation of the polyunsaturated fatty acids as discussed in Section 3.2 of chapter 5. The free fatty acid (FFA), free water, and sediment content are also very important factors which determine the required pre-treatment for upgrading the oil to biodiesel and stability in biofuel applications. The SC-CO_2 oil has the least FFA, water

and sediment content (Table 6.4a) compared to MFM and soxhlet methods. These factors make SC-CO_2 a higher quality fuel oil for use in heating oil/power applications and conversion to a biodiesel. The high impurities and other undesired compounds make the MFM oil denser (991 kg/m^3) than the SC-CO_2 (905 kg/m^3) (detail discussion in Section 3.7 of chapter 5). However, the heterogeneity of MFM oil is responsible for its non-Newtonian and pseudo plasticity (n = 0.69) which translates to better flow properties than the more homogenous SC-CO_2 oil (n = 0.99).

Regarding recoverability, the soxhlet method has the highest efficiency (53%) compared to SC-CO_2 (48%) and MFM (21%). The overall environmental impact of MFM is the lowest and so also the recovery efficiency and oil quality hence, the SC-CO_2 method could be selected over the two methods in order to strike a fair balance between environmental impact, recovery efficiency and oil quality.

Table 6.4a: Oil quality as a function of extraction methods

Methods	SC-CO2	MFM	Soxhlet
TAG recovery (%)	86	70	66
Free Fatty Acids (FFA) (%)	2.0	8.0	7.6
Sediment (%)	0.0	0.95	
Free water (%)	0.0	1.02	
Flow index at 40 ℃	0.99	0.69	
Density 40 ℃ (kg/m^3)	905	991	
Overall recovery (%)	48	21	53

Table 6.4b: Process quality rating

Process	Human health	Ecosystem quality	Climate change	Resources	Fuel quality
MFM	1	1-2	1	1	3
Soxhlet		3	3	3	2
SC-CO_2	2	1-2	2	2	1
Notation: Good =1; Fair = 2; Bad = 3					

6.4 Conclusion

In this study, three processes to recover bio-oil from fish wastes were compared via LCA. The MFM method has the lowest overall environmental impact compared to the soxhlet and SC-CO_2 methods. This lower impact of MFM is reflected in the characteristics of all impact categories except the aquatic acidification and aquatic eutrophication (Fig.6.4) which could be due to process water discharge into oceans. However, the high aquatic acidification and aquatic eutrophication reflected a lower damage to the ecosystem quality. When considering categories of damage, the soxhlet extraction method, due to the use of solvent, has the greatest impacts on all the four categories (human health, ecosystem quality, climate change and resources). However, the oil quality produced by soxhlet method is higher from a fuel perspective than the MFM method (Table 6.4).

The SC-CO_2 method falls between the soxhlet and MFM in damage in all the four categories (human health, ecosystem quality, climate change and resources). This method requires more energy (17.62 MJ to produce 1 kg oil) than the MFM and soxhlet methods (Table 6.3b). The damage is due to emissions of gases as a result of energy and resources usage (fuel, electricity, etc.). The CO_2 recovery unit consumes the most energy input

(74%) in this process. However, the SC-CO2 method produced the best quality oil of the three methods.

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CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 Summary and Conclusion

The objective of this research was to investigate different fish oil extraction processes in an effort to produce a sustainable biofuel. Physical processing and solvent extraction are primary methods for extracting edible and inedible oils in fish processing industry. Unpurified fish oil contains a variety of impurities such as free fatty acids, primary oxidation products, minerals, pigments, moisture, phospholipids, and insoluble impurities that reduce oil's fuel quality. The amount of these impurities extracted with the oil depends on the extraction method. Series of refining processes are normally required after extraction (physical processing and solvent extraction) to remove these impurities, such as degumming, deodorization, bleaching, and neutralization. However, the operating costs associated with these refining steps coupled with loss of oil and the generation of chemical waste streams during processing increase the overall cost. The biofuel quality can be determined by the quality of the feedstock and the processing conditions, which need to be carefully managed to obtain high quality fuel. Three extraction processes (soxhlet, MFM and SC-CO_2) have been studied and the objectives were to maximize fuel quality and minimize environmental and economic costs.

This thesis is comprised of five sections:

- Literature review (Chapter 2);
- Characterization of fish oil and blend with petroleum fraction (Chapter 3);
- Solubility of fish oil in SC-CO_2 and Mass transfer modeling (Chapter 4);
- Quality evaluations (Chapter 5);

- Life cycle analysis (LCA) (Chapter 6).

7.1.1 Literature Review

This review focuses on extraction processes. An overview of developments in oil extraction processes including physical, chemical and biological processes, which was limited in the literature, was presented in this chapter. The waste derived oil for use as a fuel can be a sustainable cost effective strategy for conventional combustors, boiler engines, remote fish plants, marine vessels or in-house use. The oil product does not need to meet high purity standards associated with edible oils but must balance fuel quality with costs, process complexity and robust enough to handle different types and quality of fish residue.

The majority of advanced and modified solvent extraction methods are either designed for sample analysis or environmental studies. They have not been proven beyond laboratory and/or pilot scale. These methods are very high in extraction efficiency but major constraints are high environmental impacts (due to solvent residue) and low feedstock capacity. Therefore, the solvent extractions are impractical for large scale extraction of fuel grade oils.

The fishmeal processing is environmental friendly and relatively less energy intensive. This process has been proven to handle high volume feedstock in commercial scales. However, oil product quality is low and could be less stable during storage. Process modification to improve oil quality and to reduce further product degradation during

processing has been suggested. Nevertheless, this process is simple and has been widely used over the years.

The SC-CO_2, although limited in large scale application, can reduce the environmental impacts and safety issues associated with conventional solvent extraction processes. Quality fuel with minimal impurities can be achieved through this method. However, the costs of energy (e.g. CO_2 recycling, compression and freeze drying) and infrastructure associated with required high pressures need further assessment. More feasibility studies are also required to determine whether SC-CO_2 is sustainable in commercial scale.

Biological processes are environmentally friendly and safe. The major problem is that the processes required costly enzymes and the process control is very limited. The targeted product is protein concentrate. Hydrolysis of FFA and oil-water emulsion usually prevails due to long residence time and further downstream processing is also required. Therefore; the biological process condition is not suitable for quality fuel oil products.

7.1.2 Characterization of fish oil and blends

In this chapter the fishmeal process was modified and the quality of oil was improved compared to fishmeal process oil. The oil was blended with petroleum distillate (20 – 80 % wt.) and analyzed for physico-chemical, thermal and rheological properties. These analyses determined certain characteristics valuable in understanding biofuel behavior during application. The power law effectively described the flow properties of the oil, petroleum distillate and their blends as shear thinning fluid. As the fish: petroleum distillate mixture ratio increased, the flow property of the blend improves due to

decreased activation energy (flow barrier). As proposed in the literature ([2,4] chapter 3), the fish oil blend with petroleum distillates minimize CO and Sulphur emission in combustion engines therefore, the blend application provides the possibility to combat/reduce the environmental impact due to emissions characteristics of heavy petroleum distillates.

7.1.3 SFE and mass transfer modeling

The SC-CO_2 extraction method is effective in terms of recovery, quality product and environmental impact. The mass transfer rate effectively described the SC-CO_2 extraction process. For design and scale up purposes the optimum extraction condition and the controlling parameters are very important. For instance when external mass transfer is the controlling factor, as determined in the first stage of extraction (section 4.1 chapter 4), good practice for scale-up would be to keep the solvent to solid ratio constant. So when the maximum solubility of the fish oil in the SC-CO_2 is maintained, then the extraction may be completed using small amount of SC-CO_2 possible. A maximum yield is always desirable, but as overall oil content of the solid phase decrease, the cost of the extraction would increase. In many cases the extraction process in commercial operations is considered complete when 90% of the solute has been extracted. Therefore, it would be ideal to complete the extraction system when the system is operating in the first stage of extraction, which is limited by the solubility of the fish oil in the SC-CO_2. Furthermore, designing equipment to operate optimally as modeled in this chapter would require certain inputs be fixed, such as temperature, pressure, and flow rate, etc. The major

problem then is how to effectively scale-up to larger/commercial scale production. Thus appropriate scale-up factors must be determined for the process. However, it may be inappropriate to scale up all factors at the same rate as the extraction process may be constrained by certain key parameters (e.g. SC-CO_2 flow rate, solubility, solid moisture content, etc.), and others do not contribute to the desired extraction efficiency/outcome.

7.1.4 Quality evaluation

The quality of oils from the three different extraction processes (soxhlet, MFM and SC-CO_2) were analyzed. This chapter demonstrated the effect of extraction methods on chemical composition, thermal, physicochemical, and flow properties of salmon waste derived oils. The solvent extraction method as proposed in chapter 2, is only suitable for sample analysis due to low feedstock capacity therefore, the MFM and SC-CO_2 were major focus. The lipid composition analysis determined the major chemical classes of lipids.

The TAG lipid class is the major constituent and the most important compound useful for biofuels. The nature of fatty acid constituents of TAG largely determines the oil's combustion property. TAG can be converted to biodiesel and should be maximized in order to maximize biodiesel product. Compounds such as FFA, PL, AMPL, water and sediments are undesirable for quality oil and should be eliminated or minimized during extraction. In comparison to other methods, the SC-CO_2 method recovered the highest TAG content and minimized the content of all undesirable compounds. As such, SC-CO_2 oil would have fewer performance problems when considered for biofuel application.

The MFM oil exhibited better flow properties than SC-CO_2 but of low quality. The MFM oil would require further refining steps to attain same level of purity as SC-SCO_2 oil and would add to the overall process cost.

7.1.5 Life cycle analysis (LCA)

Increased biofuel production has been proposed as one of the solutions to the need to reduce emissions of greenhouse gases ([2,4] chapter 3). Biofuel production and implementation has yet to reach its full commercial potential, especially in the developing countries. Besides technical barriers, there are several non-technical limiting factors which impede the development of biofuel such as feedstock price, production cost, fossil fuel price and environmental impacts of production processes. The LCA quantified the environmental impacts of three biofuel extraction methods.

From life cycle analysis results, the most effective extraction method in terms of environmental impact is the MFM method. The SC-CO_2 is in between the three methods studied while the soxhlet method impacted the environment the most.

The soxhlet method impacted all damage categories and was attributed to hexane use. The impact was very high on the resources more than every other damage categories (human health, ecosystem quality and climate change).

Important factors to be considered for suitable process could be safety, costs associated, complexity and product quality. An unsafe process would be discouraged irrespective of how cheap. Poor product quality may defeat other factors such as cost effectiveness and simplicity as more refining steps would be required.

7.2.0 Recommendations

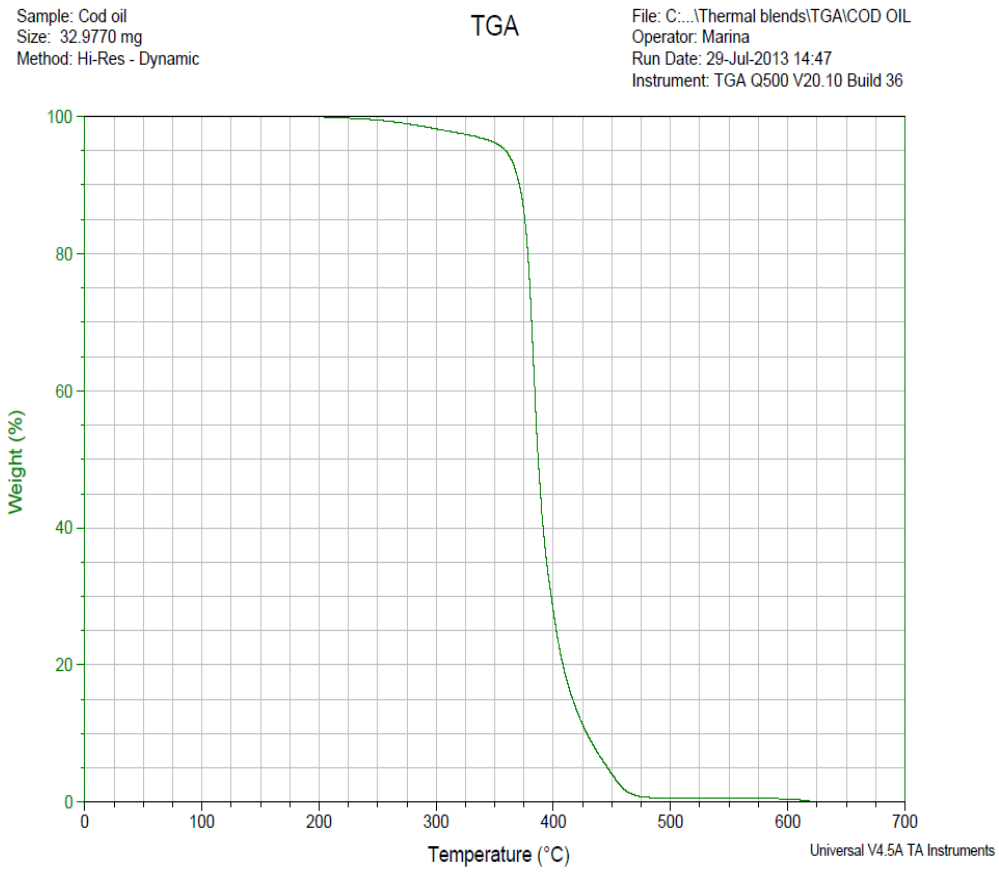
- The major part of this research provided information on a laboratory scale basis. The extraction and blending experimental were all performed at laboratory scale; further work is required to confirm the results on a pilot or commercial scale. Design parameters should be validated at lager scales for design purposes.
- The optimization of SC-CO_2 extraction process in this research was based on one factor at a time (OFAT) approach. Further work can focus on a comprehensive design of experiment (DOE) to investigate the interaction effect of two or more factors. The DOE could determine important parameters for optimum recovery conditions and also, the interaction effect of factors such as temperature, pressure, SC-CO_2 flow rates, particle size etc. could be detected with minimum number of experiments.
- The SC-CO_2 provided high quality oil as proven in this research. Further work such as stability test and blending with mineral fuel as done on the MFM oil (Chapter 3) should also be performed on SC-CO_2 oil. Further, given the more homogenous nature of SC-CO_2 oil, it may blend more effectively with petroleum based fuels.
- The LCA in this work used literature simulated energy and other inputs which could be refined with actual data based on larger scale.

- Further studies should also focus on extraction process integration into actual power plants, central heating systems, marine vessels or fish processing plants.
- Biological extraction processes such as hydrolysis and fermentation method should also be a future subject of study. Fish hydrolysate could find a good use (feedstock) in fertilizer and fish farming industries therefore a hydrolysis process could be part of fertilizer/animal feed processing steps.
- The current work does not include cost evaluation of all the processes studied. In future studies, the evaluation of the costs associated with each of the extraction process is highly recommended in order to determine their economic feasibility.

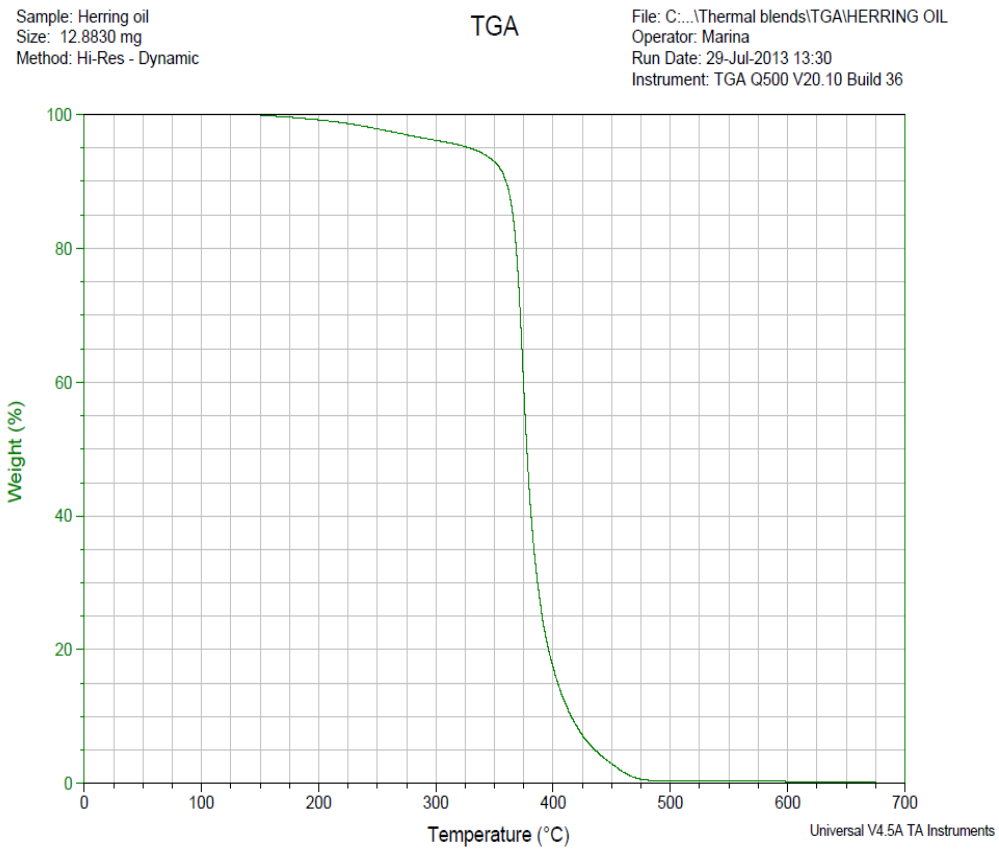
APPENDIX

APPENDIX I

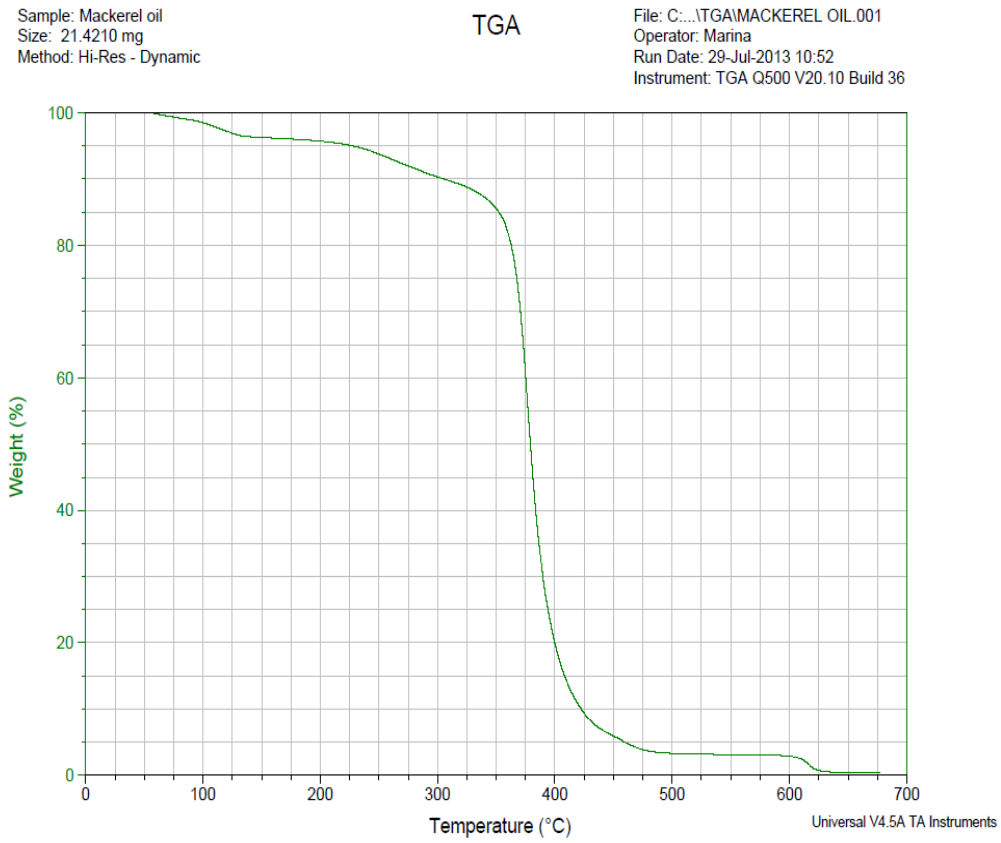
Thermogravimetric and rheological analysis of other fish species other than salmon.



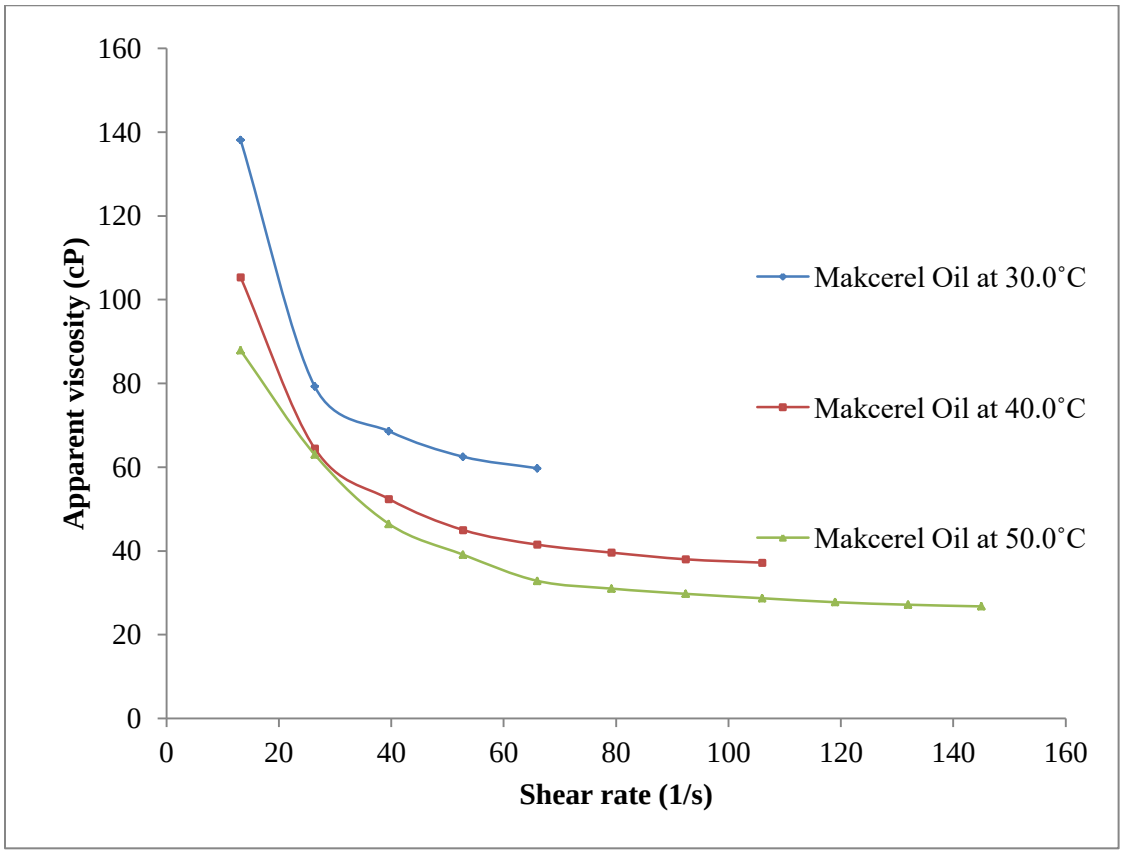
Thermal degradation of the Cod oil under nitrogen and air atmosphere.



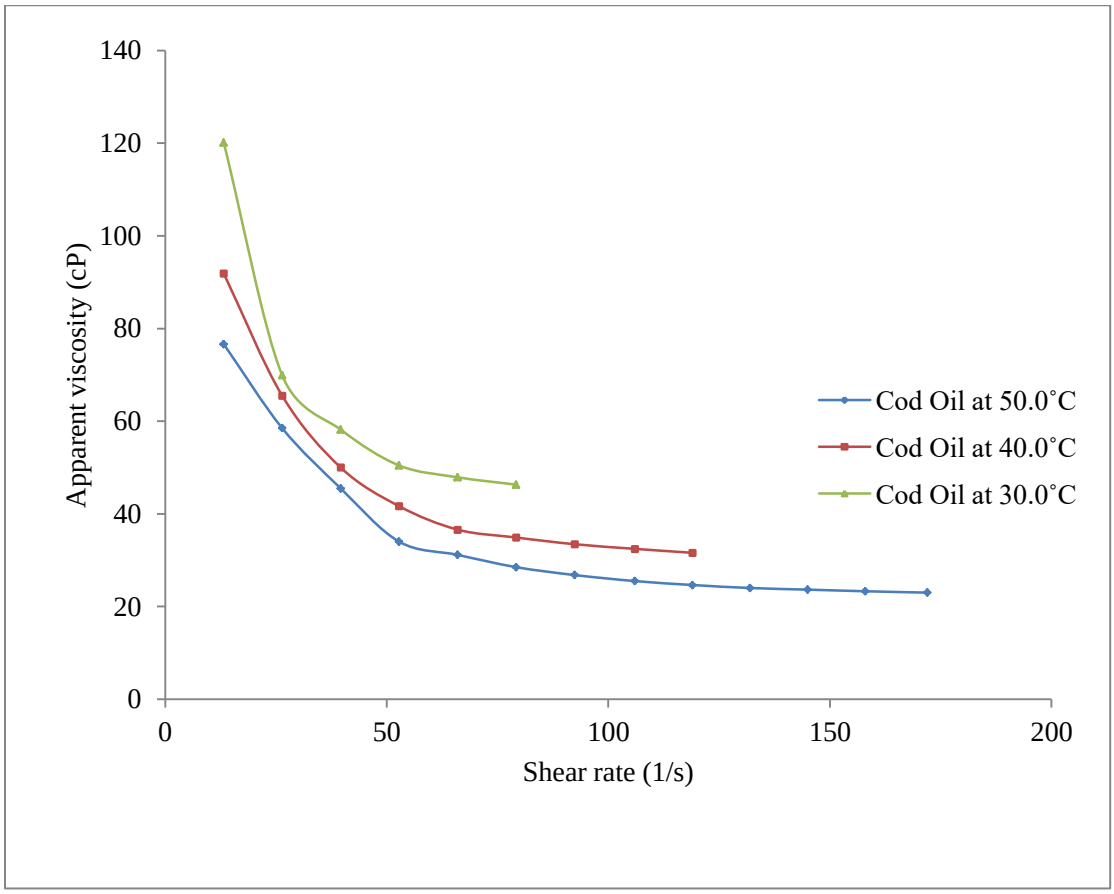
Thermal degradation of the Herring oil under nitrogen and air atmosphere.



Thermal degradation of the Mackerel oil under nitrogen and air atmosphere.



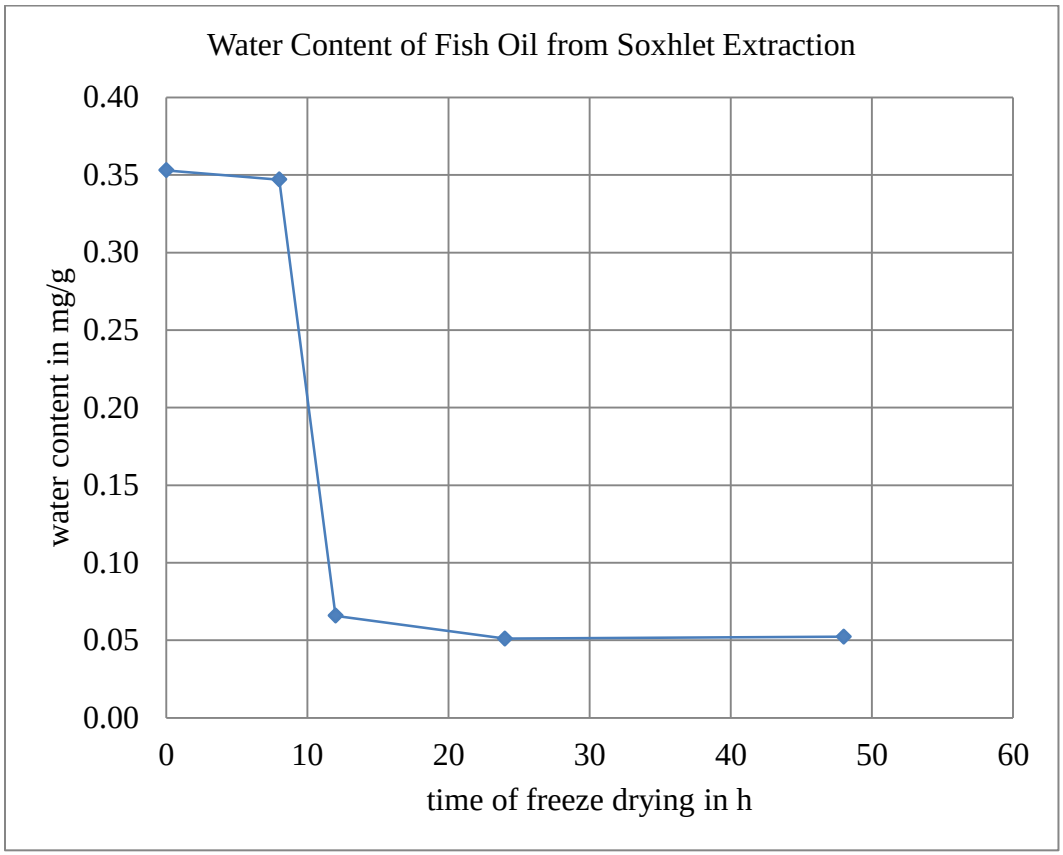
Change in apparent viscosity with shear rate at 30, 40 and 50 ^{o}C for Mackerel oils from MFM method



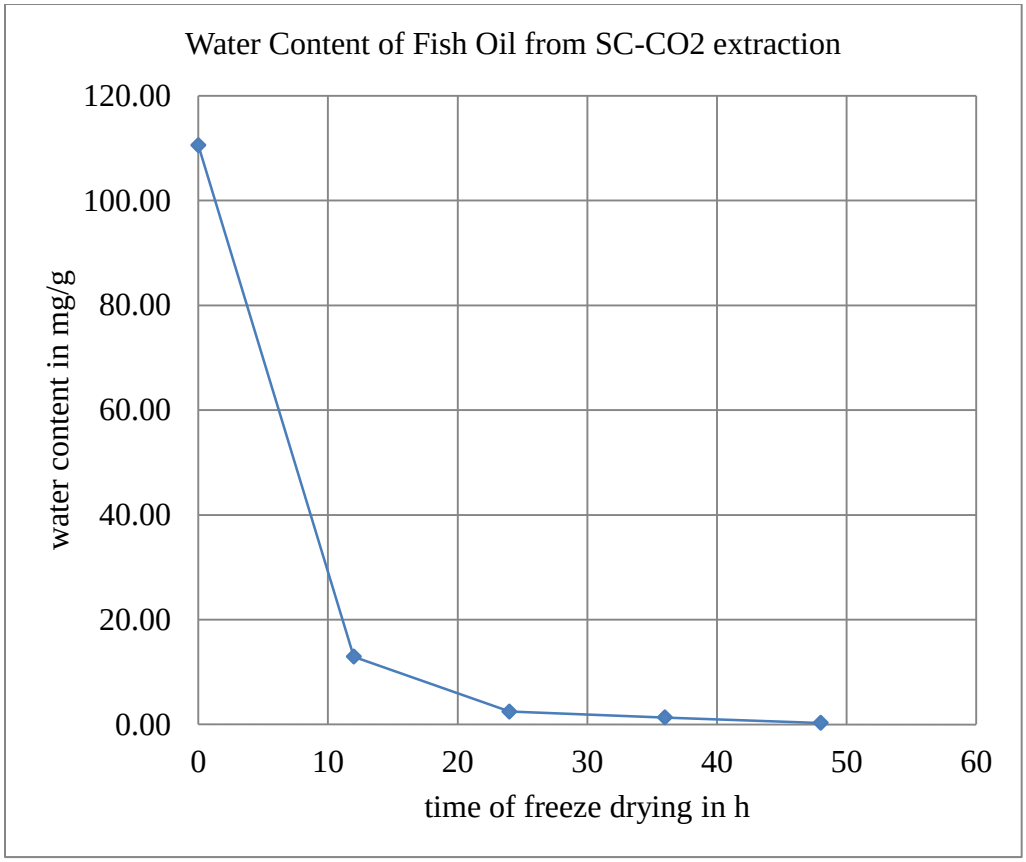
Change in apparent viscosity with shear rate at 30, 40 and 50 ^{o}C for Cod oils from MFM method

APPENDIX II

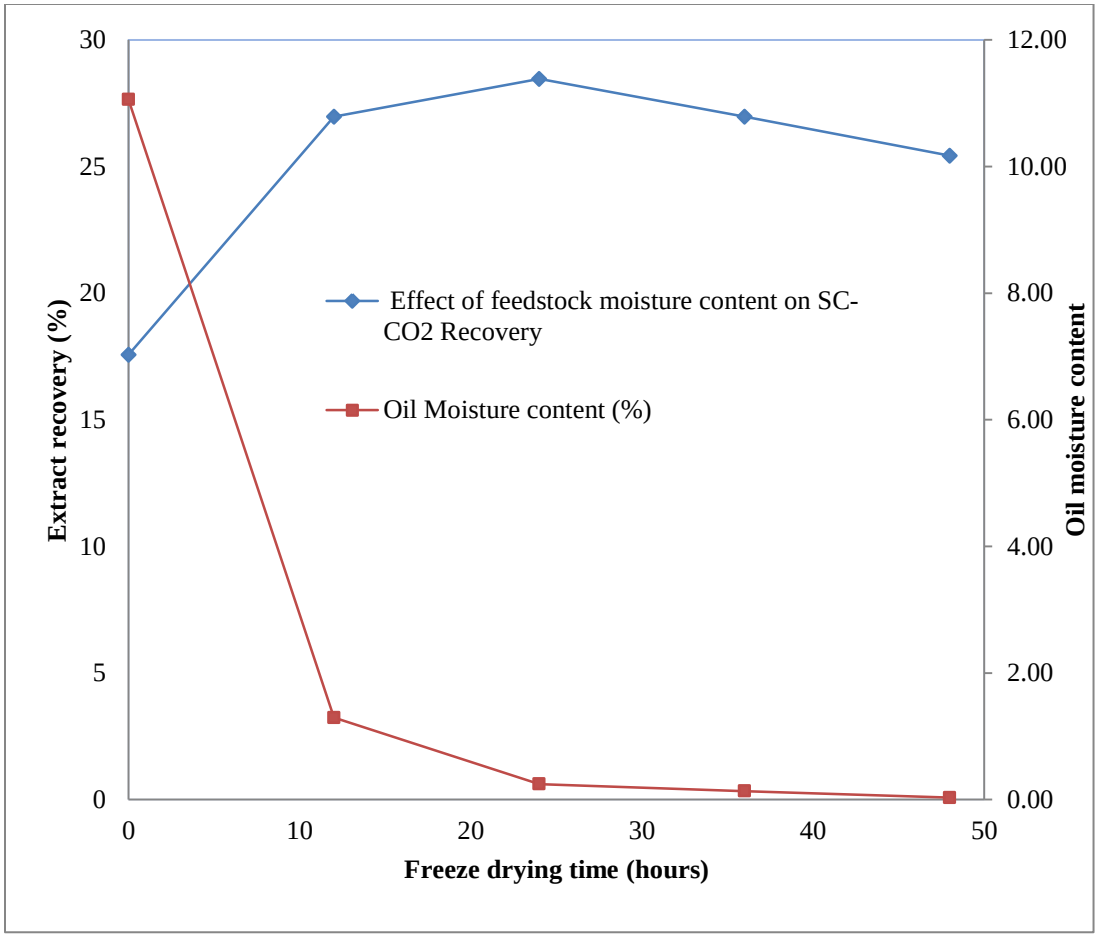
Preliminary tests on supercritical CO_2 extraction.



Effect of freeze drying time on soxhlet recovery



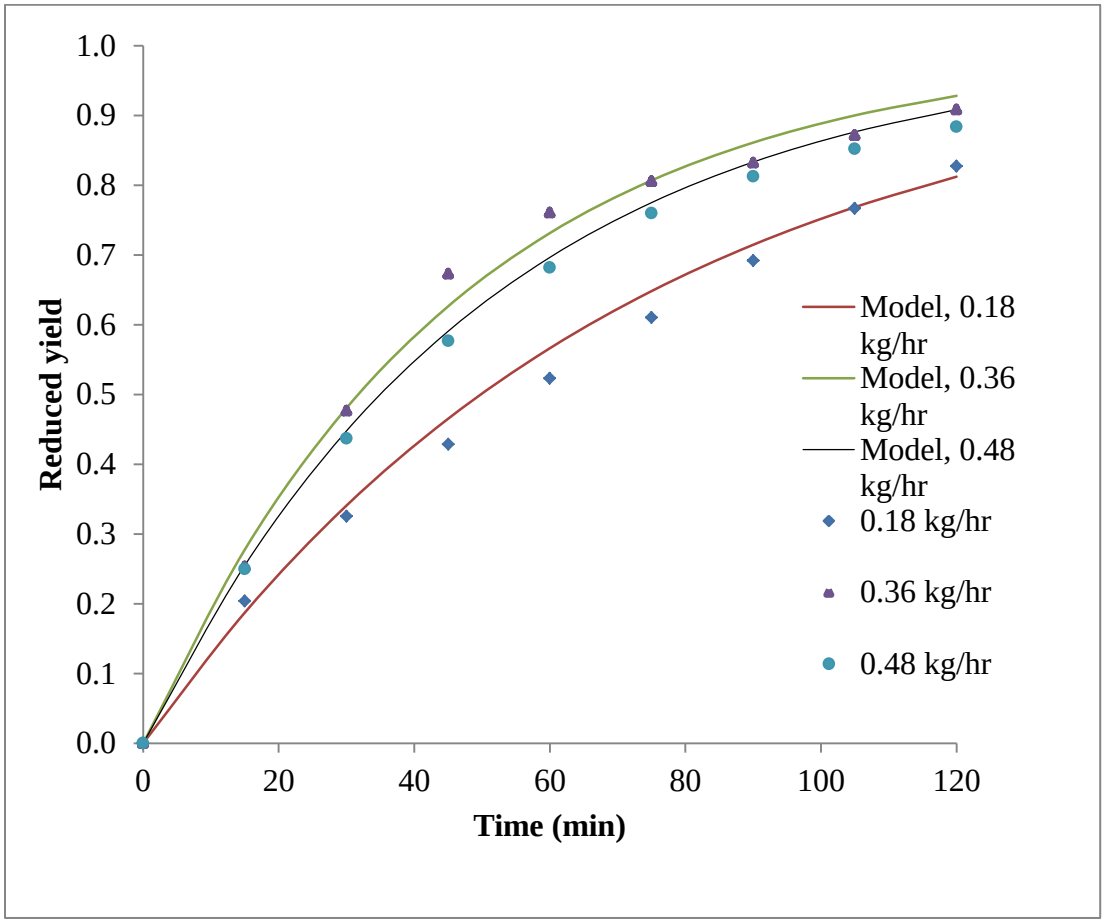
Effect of freeze drying time on SC-CO_2 recovery



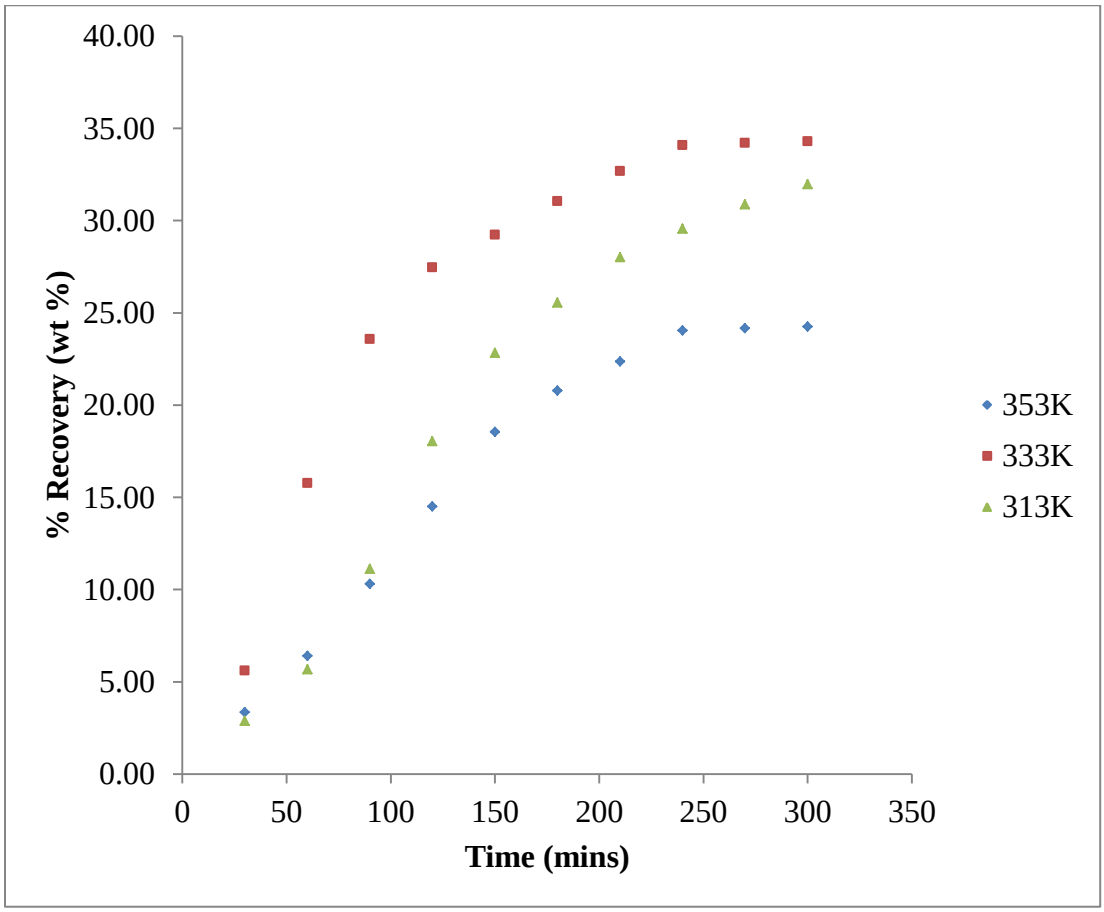
Effect of moisture content on recovery and on oil moisture content

APPENDIX III

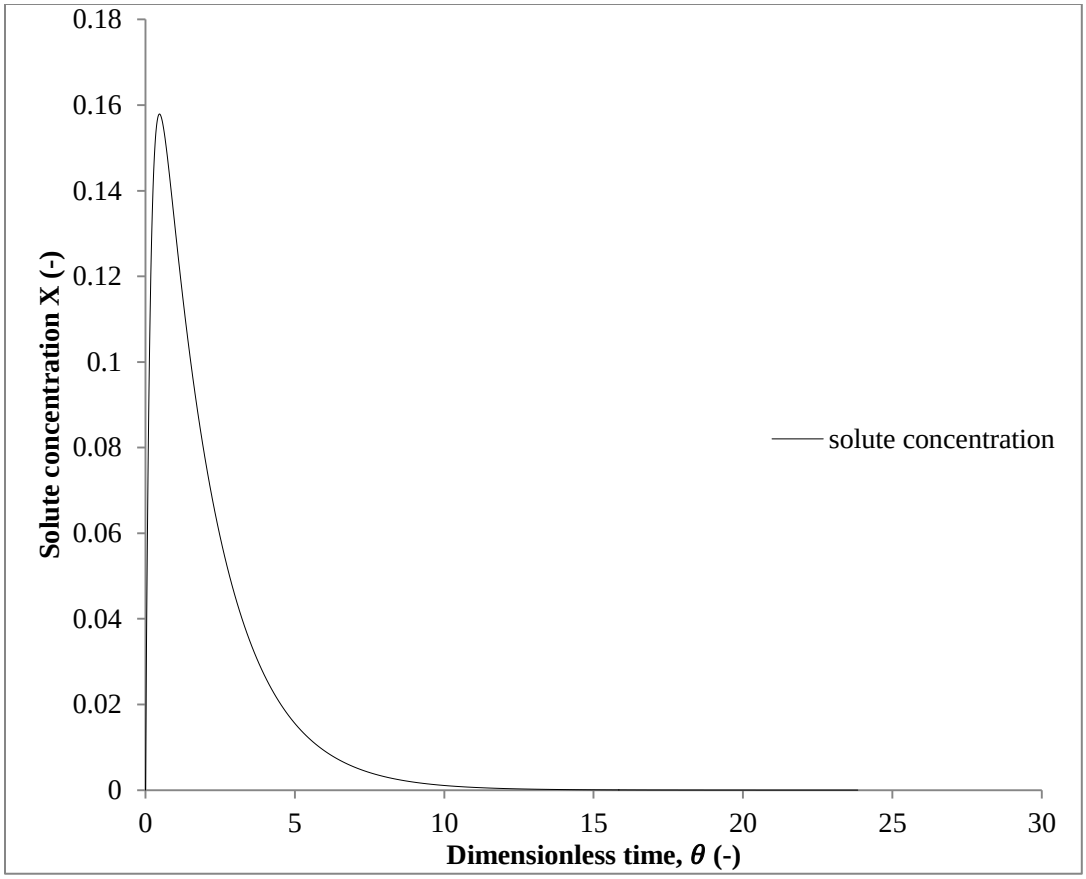
SC-CO2 model and experimental data not presented in the manuscript



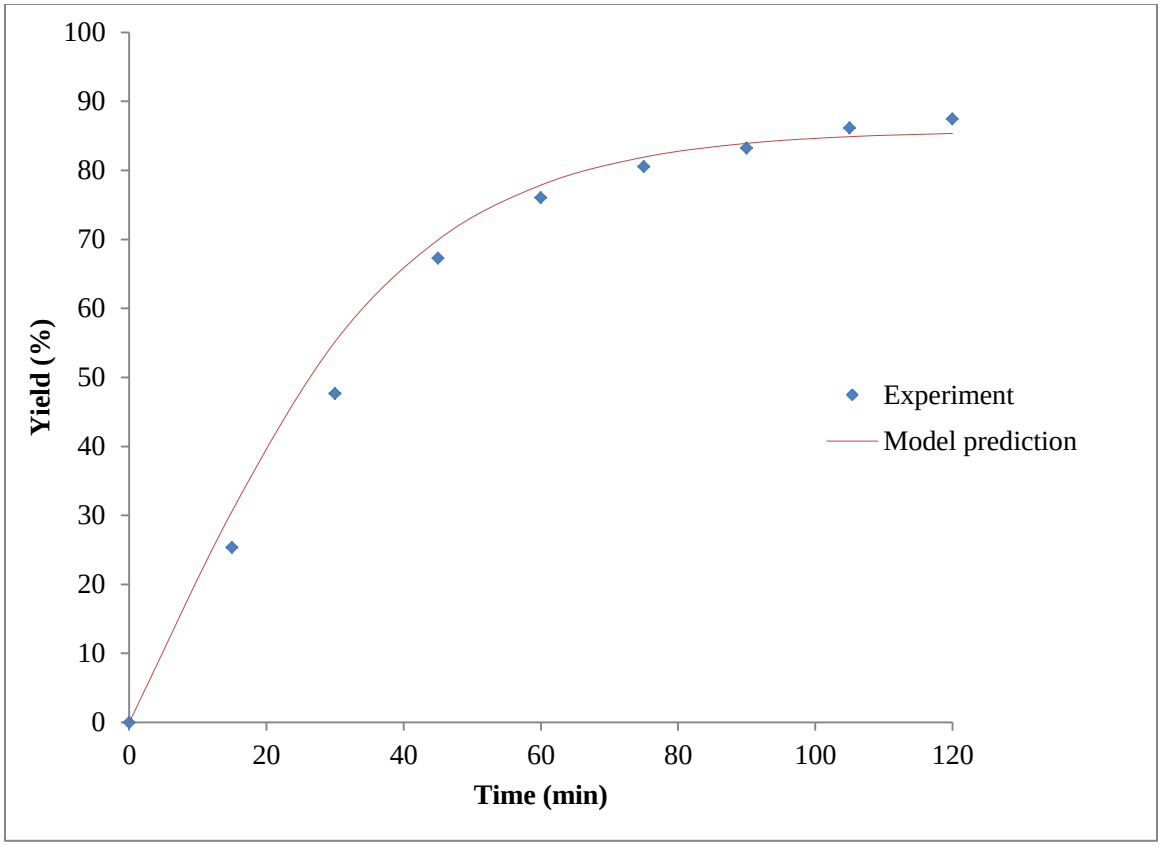
Effect of flow rate at 333K and 35 MPa



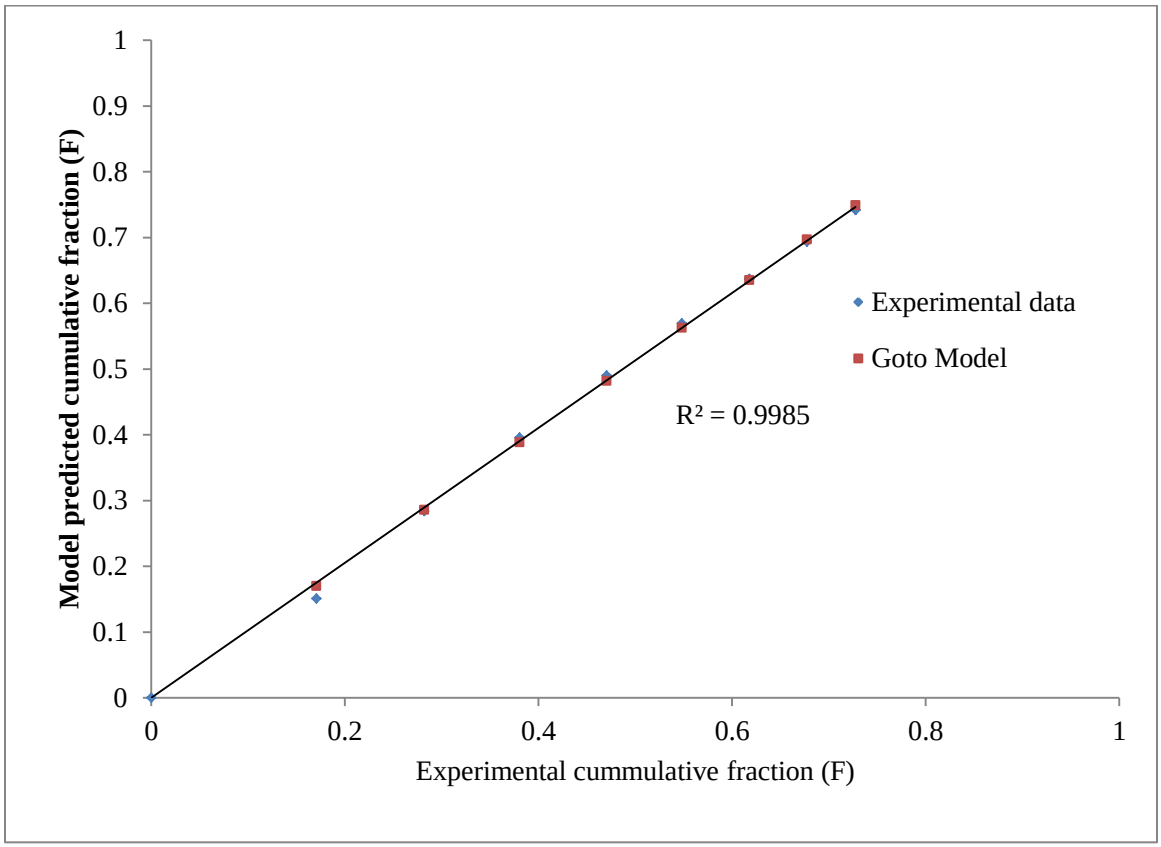
Percentage yield at 35MPa and 0.18kg/hr compared



Solute concentration model (Goto et al. 1993 model)



Percentage yield prediction (Goto et al. 1993 model) compared to experimental yield



Model yield vs experimental yield

Matlab algorithm of Goto model.

1. Mass fraction (F) as a function of time (t)

```
clc;
clear all;
De = 1.44289E-09;
dp = 4.16E-4;
ap = 7563.82;
Kf= 0.0000249;
Bi = (Kf*dp)/De;
Kg = 5*Kf/(5+Bi);
Thao =165.92;
Pha = Kg*ap*Thao;
C0 = 0.487;
t = 0;
i=1;
dt=2488.8;
while t<=19910.4;
Thetha = t/Thao;
time(i)=t;
Betha =0.463;
Alpha = 0.62;
K=3586;
C = Pha/((Betha+(1-Betha)*K)*Alpha);
b = (Pha/(Betha+(1-Betha)*K))+(Pha*(1-Alpha)/Alpha)+1/Alpha;
Gamma1 = (-b+(b^2-4*C)^0.5)/2;
Gamma2 = (-b-(b^2-4*C)^0.5)/2;
A = (Pha*(1-Alpha))/((Betha+(1-Betha)*K)*Alpha*(Gamma1-Gamma2));
X(i) = A*(exp(Gamma1*Thetha)-exp(Gamma2*Thetha));
%Thetha = time/Thao;
F1(i) = (A/(1-Alpha))*((exp(Gamma1*Thetha)-1)/Gamma1-
(exp(Gamma2*Thetha)-1)/Gamma2);
Thethat(i)=Thetha;
t=t+dt;
hold on;
i=i+1;
end
hold on;
plot(Thethat,F1,'*')
xlabel('time')
ylabel('Solute Cummulative Fraction')
```

2 Mass percentage yield (y) as a function of time (t)

```
clc;
clear all;
De = 5.91e-9;
dp = 6.8e-4;
ap = 2941.2;
Kf= 2.9e-6;
Bi = (Kf*dp)/De;
Kg = 5*Kf/(5+Bi);
Thao =104.72;
Pha = Kg*ap*Thao;
C0 = 0.487;
t = 0;
i=1;
dt=1570.8;
while t<=12566.4;
Thetha = t/Thao;
time(i)=t;
Betha =0.463;
Alpha = 0.62;
K=45.69;
C = Pha/((Betha+(1-Betha)*K)*Alpha);
b = (Pha/(Betha+(1-Betha)*K))+(Pha*(1-Alpha)/Alpha)+1/Alpha;
Gamma1 = (-b+(b^2-4*C)^0.5)/2;
Gamma2 = (-b-(b^2-4*C)^0.5)/2;
A = (Pha*(1-Alpha))/((Betha+(1-Betha)*K)*Alpha*(Gamma1-Gamma2));
X(i) = A*(exp(Gamma1*Thetha)-exp(Gamma2*Thetha));
DensityCO2 = 770;
DensityOil = 892;
y1(i) = (Betha/K+(1-
Betha)).*(X(i).*DensityOil./DensityCO2).*A.*(exp(Gamma1.*Thetha)-
exp(Gamma2.*Thetha));
X1(i) = DensityCO2*y1(i)/C0;
Q = 1.731e-7;
m(i) = ((Betha/K)+(1-
Betha)).*X1(i).*DensityOil.*Q.*A.*Thao.*(1./Gamma1.*(exp(Gamma1.*Thetha
)-1)+1./Gamma2.*(1-exp(Gamma2.*Thetha)));
Thethat(i)=Thetha*Thao;
t=t+dt;
hold on;
i=i+1;
end
%plot(time/60,m)
hold on;
for i=1:length(m)
F1(i)=sum(m(1:i))*(10000000/15);
end
plot(Thethat/60,F1,'*')
xlabel('time (min)')
ylabel('Yield (%)')
```

Supercritical CO_2 physicochemical properties

	NIST database (http://webbook.nist.gov/chemistry/fluid/).		
T (K)	P (MPa)	Viscosity (Pa.S)	Density (kg/m3)
313	15	0.000068	781
	25	0.000088	875
	35	0.000100	905
333	15	0.000047	610
	25	0.000069	785
	35	0.000084	860
353	15	0.000033	430
	25	0.000056	680
	35	0.000070	785

Critical properties of Triolein and CO_2 (simulated from HYSYS)

Property	Triolein	CO_2
Molar mass (kg)	885.4	44.01
Critical temperature (°C)	680.9	30.95
Critical pressure (kPa)	360.2	7.37
Critical mass density (kg/m^3)	143.1	467.7
Critical molar density (kg/m^3)	6.187	0.094
Critical volume ($m^3/kmol$)	3.09	0.0939